

GEOLOGY

Project title: Volcanology and Petrology of the Yellowstone Plateau Volcanic Field

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Objective: To understand the origins and eruptive mechanisms of late Cenozoic volcanic activity in the region of Yellowstone National Park and to complete systematic geologic-mapping studies carried out intermittently in the park region since the 1960s.

Findings: No new work was done on this project in 2001. USGS Professional Paper 729-G has been published and contains the principal results of this work.

Project title: Preservation of Organisms in Sinter, and Study of Sinter Textures

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Objective: To compare the preservation of plants and arthropods in sinter deposited by Yellowstone hot springs, with fossil preservation in the Early Devonian Rhynie chert of N. Scotland.

Findings: Closely comparable textures have been recognized in the Early Devonian Rhynie chert and Yellowstone sinters. These observations are used as a modern analogue for our project work on the Rhynie chert. The work suggests that the fossils in the Rhynie chert were rapidly silicified in relatively cool (<50°C) Si-rich hot spring waters.

Project title: Quaternary Geology and Geo-ecology of the Greater Yellowstone Area

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Objective: To document and determine the Quaternary geology and history of Yellowstone, the relation between Quaternary geology and ecology of Yellowstone, and the neotectonic features of Yellowstone, including faulting, and caldera unrest based on Yellowstone Lake and River level changes.

Findings: The landscapes of the Greater Yellowstone ecosystem (GYE) are shaped by geologic processes of volcanism, faulting, and uplift, all of which we associate with the Yellowstone hotspot. As the North American Plate moved SW, hotspot volcanism progressed NE and arrived at Yellowstone 2 Ma. Thousands of feet of recent uplift of the GYE have resulted in ongoing erosion of deep, steep-walled valleys in readily erodible rock.

Modern and Pleistocene weather and resultant vegetation patterns strongly relate to hotspot topography and its Snake River Plain track. Moist Pacific airmasses traverse the Snake River Plain and rise onto the Yellowstone Plateau and adjacent mountains to produce deep snows, and east of the mountains, a precipitation shadow. Such deep orographic snows produced extensive Pleistocene glaciers that covered the core GYE and produced many of the landscape features on which modern soils have formed, as well as outwash gravels (commonly covered with sagebrush–grassland) and silty lake sediments (commonly covered by lush grassland such as Hayden Valley).

Rhyolitic hotspot volcanism constructed the Pleistocene Yellowstone Plateau. Streams eroding the steep edges of this plateau form scenic canyons and waterfalls. Rhyolite is poor in nutrients and forms sandy, well-drained soils that support the monotonous, fire prone, lodgepole pine forest of the Yellowstone Plateau. Older andesite and other rocks surround this plateau and support more varied vegetation, including spruce fir and whitebark pine forests broken by grassy meadows. Upwelling waters heated by hotspot magmas drive Yellowstone's famed geysers, hot springs, and mudpots that provide habitat for specialized, primitive ecosystems of algae and bacteria.

Human settlement and use of the GYE reflects the hotspot processes of uplift, volcanism, and faulting. Uplift formed a remote highland from which streams drain radially outward like spokes from a hub. Humans have settled around Yellowstone along these drainages and established roads, irrigation systems, and political associations along them. Decision making involving the GYE is complicated by multiple jurisdictions athwart his hotspot highland, including 18 counties, 7 National Forests, 3 states, and 2 National Parks.

Project title: Groundtruthing Study of Aster and Aviris Remote Sensing Data for Hydrothermal Systems in Yellowstone National Park, Wyoming

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Objective: We obtained detailed site surveys and collected samples of soil and deposits of several selected hot springs and geysers in the Lower, Midway, and Upper Geyser Basins. The site surveys are being used for groundtruthing studies of ASTER and AVIRIS remote sensing data of this region. The site survey included non-invasive procedures such as land cover analysis, photography, Global Positioning Satellite (GPS) data collection, radiometric analysis (a non-contact thermometer), and field spectroscopy to aid in mapping of some of the sites. The samples are being analyzed with a laboratory spectrometer in visible to thermal infrared wavelengths and compared with the results of the remote sensing data. Through remote sensing and laboratory work, are characterizing the springs and their deposits from visible to thermal infrared wavelengths, especially focusing on the short-wave infrared and thermal regions. Comparisons of active, near-extinct, and extinct geysers are being determined. Because the hot springs of Yellowstone may be a planetary analogue, the results of this research will be applied to the detection of possible relict hydrothermal systems on Mars. The results of this study will form the basis of the Master's degree research for the PI.

Findings: AVIRIS and ASTER are powerful tools for the remote sensing of hot springs. AVIRIS, with its higher spectral resolution, allows for more detailed analyses than ASTER. ASTER is beneficial for regional and thermal characteristics of the basins, whereas AVIRIS can identify individual alteration minerals. Active and extinct springs have differences that are detectable through remote sensing and field spectroscopy. These differences are evident through various classification techniques, band ratioing, and spectral identification. Band ratios are useful for detecting hydrothermal alteration minerals, especially in the short-wave infrared wavelengths. However, unsupervised classifications cannot identify the entire extent of the thermally altered areas. Groundtruthing studies were necessary for truly accurate classifications. Comparison of field spectra from active geysers to extinct springs show significant variations and therefore should be easily mappable with AVIRIS. ASTER is proving beneficial for the mapping of the thermal areas. At the 90 m (TIR) resolution, the thermal areas can be distinguished, suggesting the potential success of THEMIS on Mars.

Status: For the samples collected, laboratory spectroscopy is ongoing and is being compared with the results of the remote sensing data and field spectral data. The Upper, Midway, and Lower Geyser Basins have distinct differences from each other that are detectable by ASTER and AVIRIS remote sensing. More extensive image processing and spectral studies are being done to map these areas and understand these differences. The results will explain the characteristics of the springs that are observed with the remote sensing data and lead to a greater comprehension of how to search for

similar features on Mars.

Project title: The Emergence of Scale-Invariant Architecture in Rimmed-Pool Carbonate Terraces: Abiotic Controls in Surface Hot Springs and Subsurface Cold Springs

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Objective: Similar morphological patterns are observed in modern calcium carbonate pond deposits precipitated from actively flowing spring systems in Yellowstone National Park, WY and in Fogelpole Cave, IL. These ponds and pools form terracettes, which are a series of pooled terraces deposited in a step-like pattern. On the front vertical face of the pond rims, there are microterraces that are centimeter-sized terracettes. Hence, the two spring environments show similar patterns on the macro- and meso-scales. The Yellowstone terracettes are composed of travertine, a freshwater hydrothermal calcium carbonate deposit. The Fogelpole terracettes are composed of speleothem, a cave or cavity calcium carbonate deposit precipitated from cold water. Both environments have calcite ice sheets, thin rafts of carbonate that float on the water surface before eventually sinking to the bottom. It is an interesting paradox how surficial hydrothermal springs can precipitate carbonate minerals in patterns similar to those observed in cold subterranean springs. The macro-scale morphology, depositional patterns and crystalline architecture are similar. Yet all other fundamental environmental parameters are different, including water temperature, local climatic conditions, discharge and rate of carbonate precipitation.

Some of the possible physical and chemical controls on terrestrial carbonate precipitation include carbon dioxide degassing, temperature, calcite saturation states, evaporation and inhibitors to precipitation. Other potential influences on the observed patterns include flow, slope, surface tension and surface irregularities. This study focuses on the first four parameters above to determine their effect on carbonate deposition in the two environments. Saturation states with respect to calcite of both the travertine and speleothem waters are calculated with a geochemical modeling program. As an additional modeling experiment, the temperatures of the two waters are made equal and the saturation states compared. Analysis of the crystal fabrics in each study site is done on different scales (i.e. macro, meso and micro) to determine if morphological similarities still appear at smaller scales. In the process of addressing the different possible drivers for the similarities and differences, this study also details the physical and chemical environmental conditions present during precipitation. Similar analytical techniques are used for the Yellowstone travertine ponds as for the Fogelpole speleothem pools to facilitate the comparison.

Findings: Rimmed-pool deposits in surficial hot springs at Yellowstone National Park, WY, and cold springs in Fogelpole Cave, IL, are compared to evaluate the role of physical and chemical

parameters in controlling carbonate precipitation. Macroscale (>0.5mm) morphology and depositional patterns of carbonate precipitation in these hot and cold springs are independent of water temperature and calcite saturation states. Conversely, microscale (<0.5mm) crystal shape, mineralogy and chemistry directly reflect the dramatically different physical, chemical and biological environmental conditions in the hot and cold springs. Both Yellowstone and Fogelpole spring waters precipitate hemispherical carbonate ponds in a terrace pattern. Yellowstone carbonates, called the travertine terraced ponds, precipitate at rates of 0.4–0.8 mm/day from 38°C spring water that is about 48 times supersaturated with respect to calcite (IAP/K = 48). Fogelpole carbonates, called speleothem rimmed pools, precipitate from 16°C water with IAP/K = 2. The much higher IAP/K values at Yellowstone are probably related to very high concentrations of calcite precipitation inhibitors, SO_4 and Mg^{2+} . Plane-light and cathodoluminescence petrographic analyses established very different paragenetic sequences for the two deposits. ESEM analysis indicates that the travertines are composed of a greater variety of crystal fabrics and have more euhedral forms than the speleothems. Size histograms show that the water Department and pond length of the terracette and microterrace crystallization patterns may be scale invariant. Precipitation in both systems is driven mainly by CO_2 degassing and not evaporation. Differences in equilibrium and observed $\delta^{13}\text{C}$ values of calcite suggest microbial respiration may influence carbonate precipitation.

Project title: Study and Monitoring of Selected Geyser Activity

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Objective: Continue monitoring of selected geysers and hot springs to determine behavior of the features, especially as to frequency and duration of eruptions. Where possible, correlate the geyser and hot spring activity with activity of other hydrothermal features or external forces or conditions such as water table, lake level (for geysers at West Thumb Geyser Basin), weather (e.g., rainfall or wind), and earthquakes.

Monitoring under this permit began in 1997 with seven geysers. The objective of the continuing study is to extend the scope of the monitoring both in terms of the number of features covered and the timespan.

Findings: Several data loggers belonging to the NPS and the author were deployed over the winter of 2000–2001. When the summer field season began the total number of data loggers was increased to approximately 25 (the actual count varied with time as repairs were sometimes necessary). Data from the loggers was collected weekly and analyzed to determine geyser eruption times and, where possible, duration.

The data files from the loggers along with spread sheets containing the analysis of intervals are presently located in the Spatial Analysis Laboratory in the Yellowstone Center for Resources. No major earthquakes occurred during the study period, so no correlation with seismological events was performed. Work

is continuing on the effects of rainfall and water levels in adjacent bodies of water on the geyser activity.

Project title: The Search for Microbial Biomarkers in Terrestrial Deposits

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Objective: The primary purpose of this investigation is to evaluate the fossilization process and the potential for a long term record of the microbial life that exists associated with hot springs and their carbonate (travertine) and siliceous (siliceous sinter) deposits. Basically, we are looking for biomarkers, indicators that microbes once existed as part of the hot spring environment. This will allow us to determine the likelihood of finding fossilized microbes in extraterrestrial bodies, e.g., Mars, and what is the most likely preserved material. For example, will we have a better chance of finding body fossils or geochemical indicators of former organisms.

In order to carry out this investigation, we have and will continue to analyze the waters from which the mineral precipitates originate as well as the solid precipitates of carbonate (Mammoth Hot Springs) and siliceous sinter (Cistern Spring, Norris Geyser Basin). It is our intent to search for mineralogical (crystal habit, size, etc.) and geochemical (major, minor, and trace elements as well as isotopic) differences between biotically induced and abiotic precipitates as well as microbial remains (bacterial body fossils, biofilms, etc.).

Findings: Our work has demonstrated that the microbes readily decay in the rock. We have sampled from cores taken through the siliceous sinter and have compared these analyses with those of modern material forming on the surface of the hot springs. Within a few meters Departmenth, samples in which macroscopic forms clearly demonstrate that microbes were present in abundance (e.g., stromatolites), have no remnants of the biochemical materials that are present in the surface materials. That is, decay of the organics occurs very shortly after the rock is formed. This unexpected result is not surprising considering the porous nature of the sinter and the hot, acidic, oxygen-rich waters that are constantly coursing through these deposits. Additionally, siliceous preservation of the microbes as body fossils becomes obscured as additional silica is deposited around these tiny fossils. These results indicate that hot spring deposits on Mars may not be the ideal site to look for evidence of preexisting life.

Project title: Mapping, Chronology, and Geochemistry of Hydrothermal Explosion Deposits in YNP

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Objective: Hydrothermal explosion craters and associated breccia deposits are commonly observed features in Yellowstone National Park. Visitors to popular thermal areas like Upper and Lower Geyser Basin, Norris Geyser Basin, and West Thumb Geyser Basin often see deep conical pools of thermal water and large rock fragments of explosion breccia littering the landscape. Each of these pools is an explosion crater, and they occur throughout the Park at all scales from sub-meter to kilometer diameters. Because hydrothermal explosions can occur at any time, they constitute a potential hazard to visitors in the Park. Mary Bay, a 3-km-diameter embayment on the north shore of Yellowstone Lake, is a major hydrothermal explosion crater complex that erupted about 10,000 years B.P. and deposited an apron of explosion breccia for several kilometers around Mary Bay. Other major explosion craters in the Park include: Indian Pond, Turbid Lake, Duck Lake, Fern Lake, Pocket Basin, Roaring Mountain, and a newly discovered site in the Sulfur Hills. Smaller hydrothermal eruptions, at Biscuit Basin, Porkchop Geyser, Seismic Geyser and other localities have been observed in recent years. Our objective is to use geologic and stratigraphic studies, geochronology (principally ^{14}C analyses of carbon), the new high-resolution aeromagnetic data, seismic data from the monitoring network in the Park, sonar imaging in Yellowstone Lake and geochemical, mineralogical, fluid inclusion studies to understand better the mechanisms of hydrothermal eruptions, the causes of such events, regional seismicity, and possible relations to recently discovered regional inflation/deflation cycles ("heavy breathing") in the Yellowstone caldera.

Findings: To date, over 60 samples of hydrothermal explosion breccia have been collected for alteration mineralogy, oxygen isotope, rock magnetic measurements, geochronology, and geochemistry analyses and interpretation. We plan to continue to examine and analyze recent, post-glacial, hydrothermal explosion deposits identified in cores and exposed around Yellowstone Lake, in addition to examining other less studied hydrothermal explosion deposits in the Park. We will continue to examine and analyze geothermal vent locations, physical characteristics of the deposits and vents and their distribution and chronology, and analyze stable isotopes and fluid inclusions to determine the deposits' temperature of formation and composition of fluid. We will examine details of these hydrothermal systems and their relation to magmatic activity, faulting, and changes in post-glacial lake levels. We also will apply AVIRIS data in conjunction with the recently acquired aeromagnetic survey of YNP to examine areas in and north of Yellowstone Lake that may be future potential sites of hydrothermal explosions. Geothermal fluid changes that are potentially mineralizing within the

600,000-year-old caldera may reflect contrasting time scales: The inflation/deflation cycles occur on a millennial time scale whereas the hydrothermal explosions occur within minutes. Changes in pressure and flow rate would dramatically change as mineralized geothermal fluids are released and confining pressures drop, which may significantly contribute to mineralization. Finally, high-resolution sonar imaging, seismic reflection, and submersible surveys in the northern, West Thumb, and central basins in Yellowstone Lake have identified sublacustrine hydrothermal craters, vents, domal structures, and gas pockets.

Project title: Mapping the Floor of Yellowstone Lake Using High-Resolution Bathymetry, Seismic Reflection, and Submersible Remotely Operated Vehicle

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Objective: Our goal is to obtain a high-resolution bathymetric map of Yellowstone Lake and unequivocally characterize many lake bottom bathymetric features such as faults, fissures, slumps, hydrothermal deposits, explosion craters, submerged shorelines, and glacial deposits. Subbottom seismic reflection profiles, combined with towed magnetometer data obtained at about 200 m line spacing, may discern zones of active, present-day geothermal fluid migration through sediments or hydrothermally altered sediments.

Identification of submerged faults, explosion craters, hydrothermal vents, and slumped structures will improve understanding of the interrelationship of these features, their causes, and influences by deeply circulating hydrothermal fluids. These surveys will also contribute to identify locations of spawning sites for introduced lake trout. The surveys give an accurate picture of the geologic forces forming Yellowstone Lake and how geology affects the aquatic biosphere.

Recent studies show the Yellowstone caldera has cycles of inflation and deflation with an amplitude of 30 meters on a millennial time scale. To evaluate this, submerged and tilted shorelines and sites for sub-bottom profiling and coring must be mapped using modern sonar imaging. Samples obtained will be dated. The underwater geologic history integrated with the exposed shoreline history will help define the chronology and shape of cycles related to inflation and deflation of the Yellowstone caldera, and also how these cycles may contribute to hydrothermal explosions.

Findings: The 1999–2001 high-resolution bathymetric, seismic reflection, and submersible surveys of the northern, central, and West Thumb basins of Yellowstone Lake (65–70% of the total lake) revealed a spectacular landscape previously unknown to the world. Large siliceous spires and hydrothermal explosion craters protrude into and pockmark the floor of the lake, an area mostly within

the 640,000-year-old Yellowstone caldera. In addition to the abundant circular, steep-walled depressions interpreted as hydrothermal explosion craters and the siliceous spires, we identified domal features containing gas (steam?) pockets, deformed sediments, and hydrothermal vents, and recent, previously unmapped faults, fissures, slump structures, and submerged older lake shorelines. SEM images of the spires sampled indicate their composition to be predominantly diatomaceous with subordinate bacteria and amorphous silica. To complement this work, groundtruthing of the surveyed area, involving a submersible remotely operated vehicle (ROV) with sampling (for solids and fluids) and photographic capabilities is being conducted. This summer (2002), we plan to complete our mapping of the lake, finishing with the South and Southeast Arms and parts of Flat Mountain Arm.

Our intent is to produce the following: 1) A high-resolution bathymetric map of Yellowstone Lake. 2) A high-resolution magnetic map of Yellowstone Lake. 3) High-resolution seismic reflection profiles. 4) Geochemistry and stable isotopic studies of vent waters and deposits from hydrothermal vent sites. 5) Thermal probe measurements at thermal vent sites. 6) SEM images, mineralogical analyses, U-series dates of collected siliceous spire samples. 7) Mineralogical analyses, thin sections, lithologic studies of sublacustrine hydrothermal explosion craters and associated deposits. 8) Geochemical and isotopic analyses of lake biota. 9) Various analyses of core samples at selected sites. 10) Fly-throughs of the lake to be shown at geologic exhibits within Yellowstone National Park and on our website. 11) An improved geologic understanding of various features (eg. siliceous spires, submerged shorelines, hydrothermal explosion craters, domes, etc.) and timing of specific events. 12) 3–5 minute video describing the survey and discoveries to be shown at visitor centers at Yellowstone National Park. 13) All products from this study will be made available to the National Park Service (YNP) as a base in making decisions regarding resource management issues.

GEOSEDIMENTOLOGY

**Project title: The Structure, Facies, and Deposition of Siliceous Sinter Around Thermal Springs:
Implications for the Recognition and Study of Early Life on Earth and Mars**

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Objectives: 1) To study the texture and structuring of siliceous sinter deposited around hot springs and geysers and to determine the physical, chemical, and biological controls on its deposition. 2) To identify and characterize the role of thermophilic organisms on sinter deposition. 3) To compare the structuring of siliceous sinter around Yellowstone hot springs and geysers with that of siliceous rocks 3.5–3.0 billion years old to determine if any of these ancient rocks represent ancient hot spring deposits and if there is any record of biological influences on their deposition. 4) To evaluate whether there are features of sinter that are diagnostic of biological influences that could aid in the possible identification of organisms in hot spring deposits on other planets, especially Mars.

Findings: Our investigations to date have emphasized the hydrodynamic controls on the structuring and morphology of siliceous sinter around alkaline hot springs and geysers in YNP. This research has been divided into two parts: 1) an investigation of low-temperature (less than 73°C) sinter facies, where microbial mats play a major role in the structuring and deposition of sinter at all observational levels; and 2) an investigation of high-temperature (greater than 73°C) sinter facies where thermophilic microbes are less obvious as mats but biofilms may play a role in mediating silica precipitation rates and influencing structuring and textures of sinter. Our results to date indicate that, within the high temperature zone, microbes may play a role in silica microstructure, but that hydronamics and evaporation control where silica is deposited and the larger-scale morphology and structuring of the deposits.

During 2002, additional fieldwork is planned for late May and June, mainly with the objective of monitoring silica deposition rates in a number of hot springs.

Project title: Educational Field Trip to Examine Hot Springs, Sinter, and Bacterial Mats,

Yellowstone National Park

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Objective: The object of this activity was to bring a group of six undergraduate and three graduate students from Stanford University to Yellowstone National Park to introduce them to research and study of bacterial mats and siliceous sinter deposits in the runout systems of hot springs and geysers.

Findings: The group visited Yellowstone from June 14 to June 19, 2001. The undergraduates undertook one-day projects, in which they mapped Octopus Spring or a spring in the 5-Sisters group, measured temperature profiles down the runout system, plotted bacterial mat types, and mapped the distribution of siliceous sinter. These projects were completed on June 15, 2001. Short reports were prepared in the evenings during their stay and handed in before we returned to Stanford. On June 16 we visited Steep Cone Spring and Sentinel Meadow to examine sinter mounds, on June 17 we hiked into Shoshone geyser basin to study Coral Pool and surrounding thermal features, and on June 18 we visited Fountain Paint Pots to examine in-place sinter growth experiments.

GEOMORPHOLOGY

Project title: Holocene and Modern Geomorphic Response to Fires, Floods, and Climate Change in Yellowstone National Park; Natural and Anthropogenic Influences on Stream Systems

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Objective: The current primary objective is to understand the combined influences of Holocene climatic and hydrologic change, forest fires, vegetation change, and beaver activity on small streams in northern Yellowstone. In concert with other paleoenvironmental studies, detailed geomorphic and stratigraphic analysis of fluvial and beaver dam sediments along with radiocarbon dating will shed light on intrinsic and climatic controls on stream systems and the long-term influence of beaver on valley floor environments of Yellowstone's northern range.

Findings: A stratigraphic section was examined in mixed beaver pond and fluvial sediments along Elk Creek just east of the Mammoth–Tower road. From 230 to 155 cm Departmenth, a silty clay to sandy, organic-rich, strongly gleyed unit suggests deposition in a beaver pond to marsh environment. A small charcoal fragment near the base of this unit dated 3560 ± 170 C-14 yr BP, and a conifer cone from near the top dated 1136 ± 49 C-14 yr BP. Sharply overlying this unit from a Departmenth of 155 to 123 cm is pebble gravel overlain by coarse pebbly sand, probably stream alluvium. From 123 to 95 cm, more bedded sediments generally fine upward into a very fine, sandy organic-rich unit with a charred twig dating 336 ± 49 C-14 yr BP. The top ~113 cm contains several thinner units, typically thin-bedded to laminated fine sand and silt, locally organic rich. Sediments above 123 cm Departmenth are interpreted as primarily beaver pond filling sediments with intermittent flood-deposited clean sands, notably a wavy-laminated sand with stoss-side preservation of bedforms from 95–79 cm Departmenth, and with some charcoal-rich units suggesting fire-induced sedimentation. Overall, the preliminary dating and interpretation of this section indicates beaver pond or marsh sedimentation for the majority of the last 3,800 calendar years, but broken by an episode of coarse fluvial deposition sometime shortly after 1,000 cal yr BP and before 330 cal yr BP. These dates bracketing high-energy deposition also contain the Medieval Warm Period of about 700–1,000 cal yr BP, but further dating is necessary to check for age errors stemming from reworked organic materials, more closely estimate ages of individual depositional events, and infer any climatic associations.

GEOPHYSICS

Project title: Absolute Gravity and Crustal Deformation in the Yellowstone Caldera

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Objective: The determination of temporal changes in gravity is important for assessing mass/density changes of volcanic calderas. Mass density changes reflect magmatic and/or hydrothermal activity and are an indicator of likelihood for eruption. We are collecting repeat absolute gravity and ancillary global positioning satellite (GPS) measurements in Yellowstone National Park to monitor the changes in gravity with respect to vertical height (free-air gravity change) of the Yellowstone Caldera.

Mass/density and vertical height changes reflect the movement of water and inflation/deflation of geysers and geyser basins. We are collecting temporal gravity and GPS datasets at individual geysers and plan to extend this to several geyser basins to address geyser dynamics.

Findings: We have acquired new data that indicates there are large temporal microgravity and ground motion signals associated with Yellowstone geysers. In October 2001 we observed a 8–10 microgal quasi diurnal signal in absolute gravity at the East Warming Hut in the Old Faithful Village at a distance of 70 meters from the Old Faithful geyser. This amount of gravity change is equivalent to 3–5 centimeters of vertical motion if there were no mass changes. We observed centimeter level variations in the ground motion at the same site with GPS data. Preliminary comparisons of observed gravity and ground motion suggest that the vertical motions are accompanied by mass changes. These observations are significant to characterizing geyser dynamics and require further data acquisition to quantify the mass motion and deformation of Yellowstone geysers and geyser basins.

In October 2001 we also revisited our baseline absolute gravity site at Canyon Village and established a new baseline site at Mammoth. We were able to complete some of the gravity gradients at our other baseline sites that we need to compare our 2000 absolute gravity measurements with previous relative gravity observations. We are planning to revisit all of our baseline sites in 2002 as well as make observations at some additional geyser basins.

Project title: Crustal Structure and Composition of Yellowstone National Park: Relation of

Crustal Structures to Geology, Hydrothermal Alteration, and Seismic Activity

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Objective: A high-resolution aeromagnetic survey flown over Yellowstone National Park shows a broad spectrum of contrasting magnetic patterns reflecting variations in rock composition, types and degree of alteration, and crustal structures. Compared with previously obtained low-resolution aeromagnetic data, which showed broad regional geologic trends, the new aeromagnetic data collected at low-altitudes with closely-spaced flight lines and integrated with geologic mapping, rock property measurements, and remote sensing data show this high-resolution data to be extremely useful in revealing small-scale geologic features. This method has identified fractures and areas of alteration that previously have not been mapped, identified the extent of individual geologic units and structures, and estimated the magnitude of hydrothermal alteration. Magnetic gradient trends follow the mapped north-south Basin and Range structural trends. These trends are at small scales such as in the hydrothermal basins and at larger scales such as with fault systems suggesting that the regional stress field localizes much of the present-day hydrothermal activity.

Objectives in this final phase will include a focus on collecting samples for rock magnetic and other physical characteristics to refine interpretation of aeromagnetic data; examination of the interrelationship of hydrothermally altered rock units, slumping soils on steep terrains, and timing of deformation; and investigation into the interrelationship of structural trends, fossil and active hydrothermal alteration, and regional seismicity.

Continue analysis of new high-resolution aeromagnetic survey over most of YNP and compare with magnetic susceptibility and remanence measurements. Collect and analyze magnetic remanence and susceptibilities of fresh and altered volcanic and sedimentary rocks. Prepare manuscripts of aeromagnetic data in terms of its usefulness in mapping volcanic flows, faults, and zones of alteration. Compare magnetic susceptibility, zones of alteration, oxygen isotopes, and total magnetic intensity of specific volcanic units. Demonstrate flow directions of ignimbrites in the Yellowstone Plateau volcanic field using anisotropic magnetic susceptibility.

Visual inspection of the aeromagnetic map with superposed geologic features suggests that there may be distinctive magnetic anomaly minima associated with mapped zones of hydrothermal alteration of the source rocks. To investigate this, we want to accomplish three objectives: 1) Quantify and verify the relationship between the lows and alteration and use the aeromagnetic data to map the extent of alteration zones beneath covered areas. 2) Analyze the aeromagnetic map for trends, which might delineate the structural fabric of older geologic structures which controlled the loci of volcanism within the Yellowstone system. 3) Map textural measures of the aeromagnetic anomaly field, which might be related to rock lithologies and thus be of use in elucidating the geologic structure. To do this, we will apply new analysis tools in conjunction with Mark Gettings (USGS,

Tucson) to the data and field check the data.

Findings: A paper describing the method and the case study will be published.

Project title: Operation and Development of an Earthquake and Volcano Information System At Yellowstone (YSGN) and Ancillary Research on the Geodynamics of the Yellowstone Hotspot

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Objective: The Yellowstone seismic and GPS networks (YSGN) operates an earthquake and GPS information system at Yellowstone National Park and surrounding area focusing on the Yellowstone volcanic field and associated fault zones. Specific tasks include operation of the 22-station seismic and five-station GPS (Global Positioning System) network station installation, maintenance, recording, processing, and analyses of data from these systems. The primary goals of the YSGN are to monitor and assess seismicity and ground deformation that may be related to both volcanic and earthquake activity. Data are provided to the National Park Service and USGS management, NPS public safety and interpretation groups, as well providing online data for scientific research for interested users.

Findings: In this report period we expanded our efforts to establish the USGS Yellowstone Volcano Observatory (YVO), a joint partnership between the USGS, the NPS and the University of Utah that provides a coordinated planning and implementation body. The USGS Volcano Hazards Program jointly funds this cooperative project with partial support from the National Park Service (NPS) for field work. The National Science Foundation provided funds for three of the GPS stations. The primary products of the project are earthquake catalogs, ground deformation information, and the services of a regional earthquake and GPS recording and information center, including timely release of unusual volcanic and earthquake activity reports to the USGS and the NPS.

The YSGN is designed to monitor earthquakes by seismic and GPS methods of the Yellowstone volcanic system, including Yellowstone National Park and the nearby Hebgen Lake fault zone. This project provides real-time earthquake surveillance by an upgraded 22-station, 32-component, seismic network telemetered to Salt Lake City, Utah that is digitally recorded at the University of Utah. Five continuously recording GPS stations provide monitoring of the crustal deformation that is focused on the caldera and related features.

Special note: Backcountry access for fieldwork in Yellowstone was curtailed due to FAA grounding of the NPS contracted helicopter following the September 11 terrorist attack. This resulted in postponement of much of our planned fieldwork for 2001 until summer 2002.

In addition to routine network operations, notable efforts under this cooperative agreement during the report period related to fieldwork as follows: Continued upgrading of seismograph stations against the harsh winter conditions of Yellowstone. This included 1) seismic and GPS instrument repairs and upgrades, 2) replacement of batteries at numerous sites, 3) repair of ice damage to radio antennas, solar panels, and cables, and 4) installation of a new digital seismic station at Old Faithful. This was done at several seismic and GPS stations and telemetry relay stations.

Assistance to the National Park Service with long-term plans for implementing volcano and earthquake hazard assessment and identifying manpower needs and preparing for data input into the new science centers at Old Faithful and Canyon and tuning up the helicorder in the Old Faithful Visitors Center.

A major effort this year was the organization of the Yellowstone Volcano Observatory (YVO). Meetings were held in Yellowstone National Park with USGS, NPS and University of Utah representatives to plan the organization and to facilitate communication between these groups. An MOU between these groups was signed in June 2001 that formalized the YVO and a web site was established for status of volcano and earthquake information, monitoring status and related information. Monthly updates of monitoring activities are posted to the YVO site at: <http://volcanoes.usgs.gov/yvo>.

Epicenters of 1,900 earthquakes ($M \geq 3.8$) were located in the Yellowstone region during the period October 1, 2000 to September 30, 2001. The seismicity sample includes two shocks of magnitude 3.0 or greater and no shocks of magnitude 4.0 or greater. The largest earthquake within the Yellowstone region during the report period was a shock of magnitude 3.8 that occurred on November 24, 2000 (04:20 UTC), located two miles north of Norris Junction, WY.

GPS field campaigns, at up to 140 points in and around the Yellowstone hotspot, were conducted in 1995 and 2000 to measure deformation of the Yellowstone volcanic system. The 2000 campaign also incorporated data from continuous GPS stations in Yellowstone and the eastern Snake River Plain. Station positions from each year were combined to obtain the station velocities for the network. In order to constrain the network to a North America fixed reference frames, coordinates of easternmost stations were fixed. These stations were assumed to be in the stable continental interior.

For the Yellowstone caldera, GPS results revealed uplift, with up to 8 mm/yr, north of the caldera and horizontal southwest extension of 6 mm/yr. The uplift represents a reversal in the direction of vertical deformation from the earlier decade, 1985 to 1995.

The University of Utah personnel published ten papers using data from the Yellowstone Seismic and GPS Network.

HERPETOLOGY

Project title: Amphibian and Reptile Inventory and Monitoring: Grand Teton and Yellowstone National Parks

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Objective: 1) Systematic surveys: Survey potential amphibian breeding habitat in randomly-chosen watershed units (7th level Hydrological Units); identify locations of active amphibian breeding sites and presence of amphibian and reptile species. 2) Targeted species surveys: Visit a subset of previously identified boreal toad breeding sites to determine if toads continue to breed at these sites. 3) Update species lists for amphibians and reptiles documented in Grand Teton (GRTE) and Yellowstone (YNP) National Parks. 4) Conduct monitoring of a Columbia spotted frog population at a site in YNP where historical information exists and which has been monitored annually since 1991.

Findings: 1) In YNP, we worked in eight watershed units, conducting surveys at 237 sites. At 154 sites with apparent potential amphibian breeding habitat, 70 sites were occupied by breeding amphibians: tiger salamander (eight sites), boreal toad (three sites), boreal chorus frog (49 sites), and Columbia spotted frog (30 sites). Two of the boreal toad sites were in one watershed unit. 2) Boreal Toad surveys: We visited six previously-identified boreal toad breeding sites in YNP. Reproduction was documented at four sites. We found few toad metamorphs, relative to previous years. Drought conditions reduced habitat size and quality. 3) Amphibian species found in YNP in 2001: Blotched Tiger Salamander (*Ambystoma tigrinum melanostictum*), Boreal Toad (*Bufo boreas boreas*), Boreal Chorus Frog (*Pseudacris maculata*), Columbia Spotted Frog (*Rana luteiventris*). Reptile species found in YNP: Northern Sagebrush Lizard (*Sceloporus graciosus graciosus*). Wandering Garter Snake (*Thamnophis elegans vagrans*), Prairie Rattlesnake (*Crotalis viridis viridis*). A database with all amphibian and reptile observations and a report were submitted to the NPS GRYE Inventory Program. 4) Columbia spotted frog population monitoring: There were 13 egg masses in the main study area and 30 in a downstream area that is monitored less intensively. This is slightly lower than last year. Population estimates based on photo identification are pending. In 2001, we found that 25% of the frog population in the main study area was in the juvenile life stage, compared to 52% in 2000. This reduction probably reflects recruitment failure in 2000, which was a drought year. In 2001, the drought continued and very few tadpoles survived to metamorphosis in the main study area; we thus expect to see continued decline of the juvenile component of the pop-

ulation in 2002. We also predict that winter mortality may be high this winter (2001–2002) due to low water levels in the springs and streams where the frogs hibernate.

Habitat use patterns (based on frog distribution as revealed by the capture surveys) indicated that frogs continue to avoid the portion of main study area that is within a horse pasture, where stream banks and stream-side vegetation have been apparently affected by grazing. However, this stream section is also directly below the outflow from a water chlorination/pumping station, which may also affect habitat. Frog use of the forested area most heavily disturbed by recent fuel hazard reduction was also scant, although one frog was found under reintroduced woody debris. Improvements of the fence around the breeding pool by district resource management staff appeared to help improve habitat quality and extend water retention. Spotted frog breeding attempts at a wetland adjacent to the residential area (which we think is a relatively new breeding site) failed when the wetlands dried up in early August. However, spotted frogs did breed successfully at the downstream monitoring site, and some of the recruits were found dispersing upstream in the fall; possibly they will add to the frog population in the main study area. We located an over-wintering site in a small spring. This site appears to be used only by young-of-the-year. Discovery of this over-wintering site is significant because very few spotted frog hibernacula have been identified in the GYE.

HYDROLOGY

Project title: Rocky Mountain Snowpack Chemistry Monitoring

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Additional investigator: John Sacklin, Craig McClure

Objective: 1) Long-term monitoring of annual snowpack chemistry on and off snowpacked roadways where snowmobiles travel. 2) Compare levels of acidic precursors, especially ammonium and sulfate, in snowpacked roadways subjected to low and high levels of snowmobile traffic during winter, to background levels in off-road snowpacks.

Findings: The elevated concentrations of ammonium and sulfate detected in recent years at sites near Old Faithful, especially, were seen again in the 2001 snowpack. The pattern of higher concentrations in snow in snowpacked roadways relative to concentrations measured off roadways used by snowmobiles by distances ranging from about 50 to 10,000 meters was observed again in the 2001 study. We will continue the monitoring at the same sites as in recent years during 2002 (West Yellowstone, Old Faithful, and Sylvan Lake).

Project title: Disturbance Impacts on Stream Morphology, Microhabitats, and Riparian Ecology

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Objective: The goal of this project is to determine the distribution and persistence of impacts from mining operations and fire on stream morphology, in-stream habitats, and riparian ecology.

Findings: Mine impacts persist for decades to centuries after mine closure in mountain streams like those of Northeastern Yellowstone National Park. In Soda Butte Creek, tailings dam failures emplaced tailings well above the reach of modern day floods. Modern floods therefore cannot cleanse the riparian environment of these toxic metals, which continue to impair vegetation. The alpine and subalpine environments where mining occurred do not recover over decadal time scales

under natural conditions, so high runoff continues to introduce metals to the active stream, where they impair benthic communities.

LIMNOLOGY

**Project title: Limnology Laboratory Fieldtrip: Impacts of Geothermal Inputs on a Stream
Ecosystem: the Firehole River**

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Additional investigators: Michael C. Marshall

Objective: The objective of our field trip was to expose students to basic stream sampling approaches and examine how geothermal inputs structure river ecosystems. Students write a long report in the form of a scientific paper. I hope to continue this project for the foreseeable future, as it will contribute a small amount of stream monitoring each year for two sections of the Firehole River.

Findings: We used two study sites for the research, the Firehole River above the upper Geyser Basin, and the Firehole River upstream of Ojo Caliente, where it is hottest. We measure invertebrate abundance, algal biomass, discharge, geomorphology, temperature, alkalinity, pH, and ammonium concentrations. Alkalinity, pH, temperature, and algal biomass are higher at the hot site than the cold site. Invertebrate taxa richness is lower at the hot site, and we find exotic New Zealand mud snails there.

Project title: The Biogeochemistry of Sublacustrine Geothermal Vents in Yellowstone Lake, WY

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Objective: The overall objective is to develop a quantitative understanding of the biogeochemical impacts of hydro-geothermal activity in Yellowstone Lake. This includes 1) exploration and discovery of the distribution and geochemistry of hydrothermal vents within the lake using submarine

technology in the form of remotely operated vehicles (ROV's) and in situ instrumentation; 2) quantification of biogeochemical fluxes and budgets; and 3) determination of the influence such fluxes have upon the biology of the system and the relative importance of photosynthetic, heterotrophic and chemolithotrophic production.

Findings: Mineral inputs to Yellowstone Lake, WY come from a variety of sources, including hydrothermal vents, ground water, rainwater, flux from sediments and direct runoff. One third of Yellowstone Lake is directly influenced by hydrothermal activity (hot water vents and fumaroles). Geothermally heated water percolating through the chamber is highly enriched in carbonate, silicate, chloride, and methane, with some locations additionally rich in iron and sulfide.

Microorganisms that live in high temperature ecosystems are tightly coupled to their environment. A detailed understanding of the geochemistry of hydrothermal environments can be an important component in deciphering critical characteristics for the presence of microbial life under these changing conditions.

More than 25 chemosynthesis incubations included more than 20 vent samples and an array of associated water column samples. Due to weather and scheduling constraints, the West Thumb and Mary Bay areas of the lake were intensely sampled while Stevenson Island and outlying areas near Mary Bay were postponed until 2002. This permitted us to apply more detailed analyses of chemosynthesis in the two regions than had been possible in previous years. Especially improved in 2001 (but not yet optimized) was examination of high temperature chemosynthesis (50–700°C) in parallel with in situ temperature incubations.

Vent waters in West Thumb typically contained sub-micromolar concentrations of Fe while those in Mary Bay and off Stevenson Island contain up to 10M. The water column concentrations of dissolved Fe range from 250 to 450 nM in Mary Bay, but were below detection (180 nM) in the waters of South East Arm, West Thumb, and off Stevenson Island.

Pore water and vent water chemistry provide evidence for lake water dilution of vents below the sediment-water interface. Significant fracturing of source water conduits was indicated by extreme differences in pore water profiles from cores less than 5 m apart in geothermally vigorous West Thumb. Some samples approached theoretical reservoir composition for conservative geochemical tracers.

Porewater results from the geothermally active areas of Mary Bay and West Thumb show Cl-enrichments reaching several mmolar and, in the case of Mary Bay, extrapolate to the geothermal end member (~ 20 mM) at a depth of only 2–3 m. These steep concentration gradients support diffusive Cl- fluxes across the sediment-water interface three orders of magnitude higher than those in non-venting depositional areas.

Project title: Biocomplexity-Incubation Activity: Developing Conceptual and Mathematical Approaches to Model Transport and Transformation of Elements Through a Geothermal Landscape

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Additional investigators: Nancy Hinman, Patricia Colberg, Joseph Meyer, James Drever, Patrick Sullivan, Benito Chen, Jennifer Tank

Objective: The objective is to develop a research proposal and program under the National Science Foundation Biocomplexity initiative. As part of this proposal we are mining previously collected data, and collecting preliminary data, and holding workshops and meetings in Yellowstone. Our research will address interaction of hydrology, geochemistry and microbial uptake on controlling element transport and transformation in geothermal streams in the Firehole basin.

Findings: In 2001 we held a workshop in Yellowstone and collected some water chemistry data from previous Yellowstone research. We collected very preliminary data on element concentration along geothermal tributaries to White Creek and Rabbit Creek. Both streams show strong changes in pH and water chemistry that correspond to physical processes such as degassing of CO₂ and uptake of nitrogen by microbes.

MAMMALOGY

Project title: The Effects of Environmental Variability on Grizzly Bear Habitat Use

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Additional investigators: Robert Garrott, Charles Schwartz

Objective: The overall design of this project is to utilize existing data, expertise and newly collected data from advanced technologies to evaluate the impact of anthropomorphic influences on grizzly bear (*Ursus arctos*) habitat selection. Initially, this study will have three areas of emphasis: 1) To evaluate potential relationships between habitat use and road density. 2) To evaluate potential relationships between habitat use intensity and types of human activity. 3) To evaluate potential relationships between habitat selection and land management status.

Findings: During the 2001 field season the Interagency Grizzly Bear Study Team and the Wyoming Game and Fish instrumented nine bears for this project. Of the nine grizzly bears collared, four were adult females, four were adult males and one was a sub-adult male. The first collar was deployed on May 7, 2001 and the last collar was deployed on July 9, 2001. These collars have a programmable duty cycle, which we programmed to attempt a location collection every 210 minutes. The collars will power down on November 15, 2001 and come back on April 15, 2002. The collars also have a remote release mechanism, which will automatically release the collar throughout the 2001 non-denning season. Collars will be collected and data downloaded by the researchers. Also during the 2001 field season we retrieved nine collars that were programmed to release during the 2001 non-denning season. Of these nine collars five were from males and four were from females. The retrieved GPS collars had a mean acquisition rate of 68% with a maximum acquisition rate of 78% and a minimum acquisition rate of 54%. The mean operational days of the retrieved collars was 260 days with a minimum operational days of 166 and a maximum number of operation days of 351. The mean number of successful locations was 1,063 with a minimum number of successful locations from one collar being 443 and the maximum number of successful locations from one collar being 1,618. In addition to GPS locations an attempt was made to locate and record locations of these bears using radio telemetry approximately every ten days.

In addition to the above activities we updated ancillary biophysical data sets, developed protocols for GPS data and began preliminary data analysis.

Next year the researchers plan to use this same collar technology and attempt to deploy 14 more collars.

In addition to the collection information on Grizzly Bears the researchers will continue preliminary

data analysis, test the affect of terrain on location acquisition success and update the current grizzly bear habitat coverages and other biophysical data layers with available data.

Project title: Population Dynamics of the Yellowstone Grizzly Bear

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Additional investigator: Mark Haroldson

Objective: To describe the population trend from threatened status to recovery and approximate stability.

Findings: These data include information collected by the Interagency Grizzly Bear Study Team (members include USGS-BRD, YNP, WYDGE, IDFG, MTDFWP, USFWS, USFS) for the entire Greater Yellowstone Ecosystem. Data obtained within YNP is not broken out separately. 63 individual grizzly bears were captured a total of 73 times during the 2001 field season in the Greater Yellowstone Ecosystem (GYE). 40 captures were new individuals that had not been previously marked. 32 captures of 28 bears were the result of management trapping efforts. 15 of these instances resulted in relocation of the nuisance bear(s). A total of 933 aerial radiolocations were obtained from 82 individual grizzly bears radio-monitored during all, or a portion of the 2001 field season. 31 of the grizzly bears radio-monitored were adult females. Two rounds of observation flights were conducted as part of our effort to count unduplicated females with cubs-of-the-year and document distribution of females with young (cubs, yearlings, or two-year-olds). The first round of flights began June 19. 79 grizzly bears were observed in 49 groups during 72 hours of flying. The second round of flights began on July 17. A total of 171 grizzly bears in 120 groups were observed during 72 hours of flying. 37 females with young were observed during observation flights; 20 of these were initial observations of unduplicated females with cubs-of-the-year. 42 unduplicated females with cubs were identified during 2001. A total of 78 cubs were observed during the initial sightings of unduplicated females. 13 single cub litters, 22 litters of twins, and 7 litters of triplets were observed. Mean litter size was 1.9. Unduplicated females with cubs were observed in 14 of 18 Bear Management Units (BMU) within the grizzly bear recovery zone. Females with young (cubs, yearlings or two-year-olds) were documented in all 18 BMUs. We documented 20 known and one probable human-caused grizzly bear mortalities in the GYE during 2001. 19 human-caused mortalities, including six adult females and eight total females, occurred within the USFWS Recovery Zone and ten-mile perimeter. 16 losses were from management removals, including four bears that were initially translocated, but became involved in subsequent conflicts and were removed. 13 and three management removals occurred in Wyoming and Montana, respectively. Five natural (four known and one probable) mortalities were documented. Six grizzly bears that died from unknown causes were documented during 2001.

Project title: Development of Aerial Survey Methodology for Bison Population Estimation in Yellowstone National Park

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Additional investigator: L. Lee Eberhardt, Steve Hess

Objective: The overall objective of this study is the development of aerial survey methodology for statistically rigorous estimation of the bison population in the Yellowstone area that will have sufficient power and precision to detect demographic trends. This methodology will allow NPS resource managers to conduct aerial surveys that provide scientifically defensible population estimates to address ecological conditions and epidemiological management issues of Yellowstone bison.

Findings: We developed survey protocols, data recording procedures, a geographic framework, and seasonal stratifications based on our aerial surveys of bison in Yellowstone from February 1998 until September 2000. We examined the reliability of the framework and strata using long-term data from 1970–1997 for both summer and winter. We used simulations to evaluate different sample survey designs and compared them to single and multiple high-effort censuses of well-defined areas traditionally occupied by bison. We found that sample survey designs require extensive information on the current spatial distribution of bison and therefore do not offer any substantial reduction in time and effort over high-effort censuses. They may also provide biased or imprecise estimates when bison are highly aggregated in summer. We used concurrent intensive ground surveys, or “double sampling”, in the Madison-Gibbon-Firehole areas and the Northern Range in winter, and Hayden Valley in summer to estimate the magnitude and variability in detection probability during aerial surveys. We calculated Lincoln-Peterson estimates of detection probability for groups of bison, and also developed a logistic regression model for detection probability that accounts for group size distribution and habitat. In winter, we found that 74.8% of the groups were detected on average from aircraft, although 91.9% of individual bison were detected, while in summer, 79.1% of groups and 96.7% of individual bison were detected. We also used photography to quantify the bias due to counting large groups of bison accurately. We found that undercounting increases with group size and can reach 15%. We compared survey conditions between seasons and identified optimal time windows for conducting surveys in both winter and summer. These windows account for the total area bison occupy, their group size distribution, and the habitats they occur in. Bison become increasingly scattered over the Yellowstone region in smaller groups and occupy habitats that are unfavorable as winter progresses. Therefore, the best conditions for winter surveys occur early in the season. In summer, bison are most spatially aggregated and occur in very large groups, primarily in open habitats in Hayden Valley, Lamar Valley, and on the Mirror Plateau in late July and early August. Our results show that low variability between surveys and high detection probability provide population estimates with an overall coefficient of variation of roughly 8% and have high power for detecting trends in population change. We demonstrated how population estimates from winter and summer can be integrated into a comprehensive monitoring

program to estimate annual growth rates, overall winter mortality, an index of calf production and how to relate these vital rates to climate and density. In September 2000, we completed field research and presented preliminary results at a national conference of The Wildlife Society in Nashville, Tennessee. We have completed a draft final report and circulated this document on January 3, 2002 for comments.

Project title: Black Bear Demographics in Yellowstone National Park: Their Interrelationship to Other Carnivores, Habitats, and Humans

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Additional investigators: Mark Haroldson, Kerry Gunther, Glenn Plumb

Objective: To determine patterns of habitat use, food habits, activity patterns, movements, and home range size for a sample of randomly captured black bears.

Findings: No additional black bears were captured during the 2001 field season. One Global Positioning System collar released as scheduled during 2001 and was retrieved. This collar contained 959 locations and had been worn by an adult female black bear. Attempts will be made to handle the remaining two black bears wearing GPS collars that did not release in dens during the winter of 2002.

Project title: Food Habits and Habitat Use of the Yellowstone Grizzly Bear

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Additional investigators: Shannon Podrutzny, Mark Haroldson

Objective: To determine habitat requirements for the Yellowstone grizzly bear and to document its return to free-ranging status.

Findings: Surveys to determine an index of spring ungulate carcass availability were conducted during May. Approximately 300 km of transect routes were surveyed in four different ungulate wintering areas. A total of 25 elk, 4 bison, 2 mule deer, and 1 pronghorn carcasses were observed for a rate of 0.11 ungulate carcasses/km. These results indicate a relatively small number of winterkilled ungulates were available to bears during the spring of 2001. Surveys of spawning cutthroat trout and their use by grizzly bears on tributary streams to Yellowstone Lake were completed in 2000. Results indicate no change in peak numbers of spawning cutthroat trout between early and recent survey periods on most tributary streams. Exceptions were West Thumb area streams where peak and duration of spawning trout had declined. Grizzly bear hair samples suitable for DNA analysis were collected near spawning streams during 1997–2000. Final results found a minimum of 75 individual grizzly bears visited spawning stream areas during the course of the study. Surveys of 19 whitebark pine cone productivity transects distributed throughout the GYE were completed during July. Mean cones per tree for the read transects were 25.5. Transects in the northern part of the ecosystem typically had higher cone production than those in the southern and eastern portions. Surveys of whitebark pine pathogens on the 19 transects were completed in 2001. Eleven transects showed definitive evidence of infection with white pine blister rust. A total of 143 grizzly bear observations, including 29 family groups, were recorded at 16 of the aggregation sites identified through 2000. Grizzly bears were observed digging in talus, presumably for moths, at an additional, previously unknown site during 2001.

MICROBIOLOGY

Project title: Survey of Naegleria

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Additional investigator: Jen Fagg, Joan Henson

Objective: Identification of thermophilic amoebae is important because of their possible impacts on human health. Recently, techniques have been developed to identify potential pathogens using modern molecular biological methods. These highly specific methods allow detection of amoebae from DNA isolated from multiple environmental samples, saving time compared to traditional isolation and culture techniques in which organisms are identified on the basis of morphology and physiology. Thus, molecular techniques allow a much more rapid and comprehensive survey of amoebae that may be present in thermal pools and are much less labor intensive. Furthermore, only small, easily collected water samples are required for processing. We propose to survey areas in YNP where people are soaking in thermal waters (either legally or illegally) for potential amoebic pathogens that pose substantial risks to humans. Information obtained from this study will fit well with the NPS goal of increasing scientific research within NPS ecosystems, inventorying species, and monitoring Park conditions. Furthermore, data obtained from this study will allow Park officials to make informed decisions regarding public health in the Park.

Findings: We began surveying areas in the park in late summer and fall of 2001. We sampled at Nymph Creek, the Boiling River, the swimming area along the Firehole River, the Madison River at the Madison Campground, and Kelly Warm Springs (in GTNP). Preparation of DNAs for PCR amplification is complete and we have begun to amplify the DNAs with taxonomically informative primer sets and to create clone libraries. We have positive PCR reactions for Nymph Creek and the Madison Campground so far, but we have not completed the analysis on all samples. We hope to complete the sequence analysis on the positive samples in the near future.

Project title: Isolation and Characterization of Thermophilic Microorganisms

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UNMC

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Objective: To determine the presence and ecological significance of thermophilic microorganisms of the greater Yellowstone Ecosystem. To obtain additional thermophilic microorganisms for microbial and biochemical studies including the purification and characterization of thermostable enzymes.

Findings: The field work in Yellowstone National Park involved a limited collection (during the last week in June) of microbial mat and water samples from previously sampled areas in White Creek and other areas as part of a long term thermophilic microbial populations study.

During 2001 collections were also made from Western Colorado Hot Springs where apparently new isolates of *Exiguobacterium* bacteria had been obtained (e.g. Pinkerton Springs seven miles north of Durango, Colorado). 16S rRNA sequence of these isolates have been deposited in the Gene Bank (Accession # AY047481) and a culture deposited in the American Type Culture Collection as *Exiguobacterium aurantiacum* var. Colo. Road (BAA-333). Reported at the 2001 national meeting of the American Society for Microbiology—Abstract I-92—Isolation and Characterization of a Gram Positive, Non Spore-forming *Exiguobacterium*-Like Organism Isolated from a Western Colorado Hot Spring.

Similar type of isolates have also been recently obtained from Southern India Hot Springs (Ganeshpuri, northeast of Bombay [Mumbai] India) and it appears that *Exiguobacterium* type isolates are also present in lower temperature Yellowstone hot springs (e.g. Huckleberry Hot Springs south of the South Entrance to Yellowstone National Park). However, for the present, the study of these and other Yellowstone microbial isolates is inhibited by not being able to deposit any proposed new isolates in the American Type Culture Collection (for study by other investigators etc.) because of a proprietary dispute between the U.S. Department of Interior and the American Type Culture Collection regarding the legal standing of such isolates of “Yellowstone Microorganisms.”

Project title: Analysis of a Eukaryotic Microbial Mat Community Across Environmental Gradients in a Thermal, Acidic Stream

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Additional investigators: Kathy Sheehan, Michael Ferris

Objective: Two Nymph Creek sites, defined in terms of differing light, pH, and temperature will be extensively monitored over diurnal and seasonal time periods. Contemporary analyses, including modern microscopic methods and rRNA sequencing will be used to document microbial diversity and analyze the mat's microstructure at both sites. Changes in macro- and micro-scale environmental conditions in the bulk water and through the vertical aspect of the mats will be recorded.

Findings: 1) We used culture-independent cloning and sequencing of PCR-amplified 18S rRNA gene segments to survey algal populations in situ. We also isolated and characterized axenic *Cyanidiophyceae* and identified them by sequencing a portion of their 18S rRNA genes. The results suggest that a strict autotrophic *C. caldarium*-like algal population is prevalent in situ. We have also determined that the algal population changes along the thermal, pH, and sunlight gradients of Nymph Creek; while the predominant alga upstream is *Cyanidium*, *Chlorella* dominates the mat at the downstream site. 2) Our microscopic examination of samples from Nymph Creek initially showed vahlkampfiids and heterolobose amoebae. This observation prompted sequence analysis of cloned rRNA genes PCR-amplified directly from Nymph Creek and revealed the presence of vahlkampfiid species. A phylogenetic comparison showed a sequence that forms an independent lineage within *N.fowleri*-like protozoa that may represent a new, potentially pathogenic species. 3) We detected sulfite and sulfate reduction by anaerobic bacteria in Nymph Creek using molecular analysis and traditional culture-based methods.

Project title: Biomolecular Diversity in Yellowstone National Park

Principal investigator: Mr. Eric Mathur

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Additional investigator: Martin Keller, Jay M. Short

Objective: Explore the microbial diversity at Yellowstone with special focus on the thermophiles that exist in Yellowstone's thermal features. Also of particular interest are microorganisms that exist

in thermal features that contain extremely low and high pH levels. The ultimate objective of our research is to discover new commercial products.

Findings: Unfortunately, we did not engage in any research activities in Yellowstone because we are still waiting for the completion of the Environmental Impact Statement.

Project title: Microbial Physiology and Ecology: DNA Damage and Photosynthesis

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Additional investigator: Rocco Mancinelli, Dana Rogoff, Dianne Purcell, Vanessa Lancaster

Objective: The objective of this project is to study diurnal patterns of organismal physiology (e.g., photosynthesis, DNA synthesis) in order to better understand evolution on early earth and the way organisms function in their environment today. Specifically, in 1999 the focus was on the effect of two naturally occurring DNA damaging agents on DNA synthesis rates, ultraviolet radiation and hydrogen peroxide.

Findings: In 2000 research focused on the effects of UV radiation and hydrogen peroxide on microbial mat communities in Norris Geyser Basin and Octopus Spring, we found that UV radiation enhances DNA synthesis rates during the day, which we interpret as being indicative of excision repair. However, previous work suggests that the damage may be due to UVA effects mediated through oxidative damage rather than the direct effect of UVB. Experiments adding hydrogen peroxide to sample showed an increase in DNA synthesis in response to small amounts of additional hydrogen peroxide, and a decrease in response to high levels, with another increase at even higher levels, about 1 mM for Octopus and Zygonium mat. At the very highest concentrations of H₂O₂, DNA synthesis, of course, drops to zero, probably an indication of cell death. For all the mats studied, DNA synthesis stopped by 1 M H₂O₂. Pre-challenging Zygonium with H₂O₂ prior to measuring the effect of H₂O₂ on DNA synthesis decreased the subsequent rate of DNA synthesis. This is suggestive of an induction of catalase. Techniques for studying levels of catalase and superoxide dismutase were begun in collaboration with Vanessa Lancaster and Bob Blankenship, Arizona State University. These studies will be repeated and extended in 2002.

The effects of several drugs were tested on the effect of H₂O₂ on DNA synthesis. Caffeine (1 mM) increased DNA synthesis in the presence and absence of additional H₂O₂ in Cyanidium, Zygonium. Zygonium mats that were placed under UV opaque screens from September to June 26, 2000 showed a down regulation in DNA synthesis when finally exposed to solar radiation in contrast to mat that was left under the UV opaque screen.

Project title: Isolation of New Hyperthermophiles and Investigations of

Hyperthermophilic Biotopes

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Additional investigator: Robert Huber, Wolfgang Eder, Christian Rudolph, Brian Hedlund

Objective: Isolation of new hyperthermophiles and investigations of hyperthermophilic biotopes.

Findings: From hot acidic soil samples, taken in 2000 in YNP, acidophilic members of the Aquificales order were enriched and isolated. This result demonstrates for the first time the existence of acidophilic Hydrogenobacter-relatives in YNP. Two publications on this topic are submitted.

During a field trip in YNP, samples from Obsidian Pool were collected for microbiological investigations.

Project title: Molecular Assessment of Microbial Communities in Hot Spring Structures and Their Responses to Light Manipulation

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Additional investigator: Evan Lau

Objective: To determine microbial community structure in lithifying structures within hot springs runoffs.

Findings: Denaturing Gradient Gel Electrophoresis (DGGE) and subsequent sequencing of partial 16S rRNA gene sequences was used to investigate the molecular biodiversity of cyanobacterial communities inhabiting various lithified morpho-structures in a hot spring of Yellowstone National Park. These morpho-structures flat-topped columns, columnar cones, and ridged cones resemble ancient stromatolites (*Conophyton*), which are presumed to be biogenic. The top, middle and bottom sections of these lithified morpho-structures, were analyzed to determine the vertical and spatial distribution of cyanobacterial communities. Results from DGGE indicate that the cyanobacterial community composition of lithified morpho-structures (flat-topped columns, columnar cones, and ridged cones) were largely similar in vertical distribution as well as among the morpho-struc-

tures studied. Analysis of partial 16S rRNA gene sequences obtained from these community profiles show that the closest relatives of these lithifying cyanobacteria are detected or isolated from hot springs. Preliminary results also indicate that the cyanobacterial communities in these lithified morpho-structures were significantly different from communities in surrounding non-lithified mats. These results provide additional support to the theory that certain *Phormidium*/*Leptolyngbya* species are involved in the morphogenesis of lithifying morpho-structures in hot springs and may have played a role in the formation of extant lithified stromatolites as well as ancient ones.

Project title: Development of Harsh Environment Biosensors

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Additional investigator: William Apel, Kastli Schaller

Objective: The major objective for this project was to develop more robust biological sensors for use in harsh environments. These environments might include high temperatures, high or low pH, or high salt conditions where conventional biological sensors fail rapidly due to denaturation of the biological component. Our approach was to isolate and purify enzymes from thermophilic microorganisms and utilize these enzymes for sensor development. These enzymes have evolved to be stable at high temperatures and would yield a biological sensor that could operate at elevated temperatures for extended periods of time.

Findings: We have been working to purify a thermostable catalase from an organism (*Thermus brockianus*) isolated from Yellowstone hot springs previously. We have partially purified this enzyme with ion exchange, hydrophobic interaction, and gel filtration chromatographies. Our initial characterization studies have shown the enzyme to have optimum activity at a temperature of 80°C and a pH of 8.0. The enzyme is active from 30°C up to at least 100°C and in the pH range from 6.0 to 10.0. The enzyme is also stable for at least seven days when incubated at 70°C. We plan to continue characterization of this enzyme including determination of the molecular weight, enzyme kinetics, and inhibitors. Future work will focus on the development of a biosensor using this enzyme.

Project title: Isolation, Identification, and Characterization of Microorganisms Living in Extreme Environments

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Objective: There are two main project objectives. First, to train students in the safe and proper sampling of hot spring environments for thermophilic microbes, as well as characterizing dissolved components in the spring waters for media development. Secondly, this study involves laboratory characterization of culturable microorganisms from the hot spring samples as well as looking at species diversity using denaturing gradient gel electrophoresis.

Findings: Sample collection and removal from the Park was not permitted under the conditions of my permit as stipulated by Ann Deutch. However, I was allowed to take students to Snake Hot Springs, Shoshone Geyser Basin, and Spray.

Samples were collected from the Bear Creek thermal area outside the Northern boundaries of the Park and characterization of the microbial inhabitants is ongoing. These springs although classified as thermal springs only have temperatures ranging from around 30–35°C degrees celsius with pH values between six and seven.

An inventory of organisms that we have identified from samples taken in previous years is being prepared for submission to Yellowstone National Park for the Thermophile Inventory database.

The status of these studies is ongoing.

Project title: Spectral Analysis of Hyperthermophile Organisms

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Additional investigators: Jeffrey Johnson, Mike Ferris

Objective: To define spectral reflectance character of hyperthermophile mat communities; determine if each community has a diagnostic spectra; determine spectral signature of the sinter; and determine if a biologic signature occurs in the sinter which persists with time. Because each organism has specific chlorophyll, bacteriochlorophyll and carotenoid compositions, each has a well defined spectral signature. Data are of sufficient spectral resolution to allow pigment identification.

A long term goal is to use spectral signatures as a reconnaissance tool to study community structure at springs that have not been examined. If, indeed, each community has a diagnostic spectra, then such data can be quickly collected at unstudied springs allowing an assessment of the organisms that are present. While this technique does not identify all organisms, it does allow a rapid evaluation of general population and any significant presence of organisms that have not been previously recognized.

Spectral data are collected field spectrometer in the range 350–2,500 nm. Mat reflectance data are collected in situ. In a few cases, small amounts of mat are removed, measured and replaced.

Typically, a fiber optic is mated to an artificial light source and placed just above the mat. This results in a field of view of a few cm. An artificial light source is used to reduce the effect of water vapor on the spectra. Because of changing cloud conditions and significant water vapor present at the springs, the spectra of natural sunlight will rapidly vary. Water vapor produces deep absorptions at several wavelength making it difficult to correct the observations. An artificial light source reduces the path length of light and therefore the influence of water vapor.

Data are collected from the source to a point where the water cools to ambient temperature. Stations are located at regular intervals on the channel or at obvious changes in the mat. This ensures that changes in the community structure are observed. At each station, the color and morphology of the mat surface are noted along with temperature and pH. Several spectra are collected at each spot and several spots are measured at each station. This avoids the problem of bad data and ensures that local variation in the spectra will be defined. In a few locations, the surface mat is scraped away allowing data to be collected for the subsurface communities. Disturbed areas which reveal underlying mat material are also examined.

In addition to observations of the mat communities, data are also collected on the silica and carbonate sinter that forms around the margin of the spring, that line the outflow channel and older materials in the immediate areas. The purpose of these measurements is to define the spectral character of the sinter and to determine the character of any biologic material that might be present but not visible. Dried, inactive sinter is also examined to determine if any organic material can be detected.

A new objective of the investigation will be to use an ultraviolet spectrometer to examine the margins and interiors of certain springs. SEM images of the sinter have revealed the presence of biofilms and microfossils, yet the temperature of the water at these locations is above that which would be expected to support life and in areas where there is no obvious mat. An ultraviolet fluorescence spectrometer that can withstand submersion into very high temperature water will be used to determine if organic material is present around the rim of the spring or lining the walls of the pool. The spectrometer illuminates the surface with an ultraviolet light source. Organics will fluorescence at specific wavelengths which can be detected by the instrument. This will allow a determination of whether organic materials are present in large quantities, which types of materials are present, and if they occur both in the pool and on the rim or only on the rim.

Findings: To date we have collected spectral data at a variety of springs spanning a large temperature and chemistry range. Sites examined include: Octopus Spring, Mushroom Spring, and various springs up White Creek above Octopus; along Nymph Creek between the source and the Nymph Lake; at several springs and channels in the Lemonade Creek area, in a number of locations in the Norris Geyser Basin both adjacent to the boardwalk and in the area around Cinder Pool; at the Chocolate Pots mounds, and several springs in the Mammoth area. In each case a series of stations were set up beginning at the source and extending down the channel to a point where the water temperature reaches ambient, where the mat disappears, or where the flow enters another flow. At each station several measurements are made to examine the extent of local variation in the spectral character.

At Octopus Spring, the *Synechococcus*, *Chloroflexus* and *Phormidium* communities each have a well defined spectral signature. The mat has been examined during two years and the results from the two sets of data are consistent. *Synechococcus* exhibits well defined absorptions at 625, 675, 740, 800 and 880 nm. *Chloroflexus* absorptions occurs at 597, 683, 747, 798 and 890 nm. *Phormidium*

has absorptions at 675, 740, 798 and 874 nm.

At a test of the ability to determine whether the spectra could be used in a reconnaissance mode, two springs (Tuft Geyser and an adjacent unnamed spring) upstream from Octopus, along White Creek, were examined. Tuft Geyser has a narrow outflow channel with a green and orange mat; an adjacent pool has a short effluent channel with a surficial green mat and an underlying red-orange mat. Spectral data indicate that the mat associated with Tuft Geyser is composed of *Synechococcus* at the high temperature end and at *Phormidium* at lower temperatures. The mat along the channel from the adjacent spring is interpreted to be a *Synechococcus/Chloroflexus* mat community similar to that at Octopus Spring.

The outflow at Nymph Creek between the springs and the lake has been examined. This spring has a very low pH (2.9). Immediately surrounding the sources there is a yellow deposit and local filaments. The yellow material is sulfur. A short distance downstream a green mat composed of *Cyanidium caldarium* occurs. Spectra of this mat indicates that while there are morphologic and visible color variations, the spectra remain largely the same. At the lower end of the stream, the *Cyanidium* is replaced by *Chlorella* which has a significantly different spectral character. Lemonade Creek has a similar acidic water supply and the same characteristics are observed there; yellow deposits around the sources are considered to be sulfur and the green mat is interpreted to be *Cyanidium*.

To test the ability to predict the biota in other acidic springs, data were collected at several spring and outflow channels in the Norris Basin in the area of Cinder Pool. Here, numerous springs occur with yellow deposits around the source and thin green mat in the effluent. Spectral data indicate these are sulfur and *Cyanidium*.

At the Chocolate Pots, the data indicate the presence of biota across the entire mound surface, even where there is no obvious mat. An absorption near 680 nm, indicative of chlorophyll is observed. The mineral material that forms the dark brown mound is ferrihydrite with the possible addition of some other iron bearing phases.

Data were collected from a number of springs and seeps in the Mammoth area to examine the biota in an environment where carbonate is mineral deposited rather than silica. These springs also contain a green version of *Chloroflexus* rather than the red version typical of Octopus Spring. These data have been collected but not interpreted.

Project title: Diversity and Habitat Range of Sulfate-Reducing Microorganisms

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Objective: Our research at Yellowstone National Park has focused on better defining the diversity of sulfate-reducing bacteria along environmental gradients of pH and temperature. Organisms having the capacity to respire sulfate drive a key step in the global cycling of sulfur and are likely an important biological presence in many of the sulfur-rich geothermal areas within Yellowstone National Park. A long-term objective is to better define the environmental limits of dissimilatory sulfate reduction. Our primary method of assessing population diversity has been comparative sequence analysis of the highly conserved dissimilatory sulfite reductase (DSR) gene. This gene can be selectively amplified from DNA recovered from site material using PCR, as reported by our research group (Wagner, Roger et al. 1998; Minz, Flax et al. 1999). Comparative sequencing of cloned DSR genes avoids the usual biases associated with culture-based methods of characterization. We complement this molecular characterization with on-site activity measurements and also use more traditional culture-based methods to evaluate cultivable sulfate-reducing bacteria.

Findings: The discovery of deeply diverging phylogenetic lineages of sulfate-reducing bacteria, as inferred from DSR gene sequence divergence, suggests that our current understanding of this important functional group of microorganisms is incomplete. Our combined analyses of different regions throughout the Park indicate that sulfate respiration is a significant biogeochemical process in many of Yellowstone's geothermal features. In 2001 we followed up on studies performed during the previous three years of the study in two established field sites and in a new study set of hot springs. In Mushroom spring we repeated experiments investigating the activity and diversity of sulfate-reducing bacteria found in the microbial mats present in the effluent channel. As observed in 2000, although these mats are relatively low in sulfate, they sustain very high rates of sulfate reduction. Molecular analyses, using PCR to selective recover bacterial 16S rRNA sequences from the Mushroom Spring mats revealed high bacterial diversity. Coincident analyses of DNA sequences encoding for the dissimilatory sulfite reductase confirmed significant diversity of sulfate-reducing bacteria in this spring as well. We also followed up on previous activity assessments at the spring we refer to as Black Sediment pool in the vicinity of Nymph Creek. Significant endogenous sulfate reduction rates were once again observed in this spring. Finally, we explored a few new sites in the vicinity of Crater Mountain and tested them for endogenous sulfate reduction activity (results pending).

Project title: Molecular Ecology of Photosynthetic Hot Spring Bacteria That Resemble *Heliothrix oregonensis*

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Additional investigators: Daniel Lodge

Objective: 1) To perform an ecological survey of thermal features in Yellowstone in order to describe the distribution of *Heliothrix*-like organisms. 2) To isolate DNA from biological samples that contain *Heliothrix*-like organisms. 3) To utilize PCR to isolate 16S rRNA genes from these DNA samples. 4) To clone, characterize, and compare representative 16S rRNA genes at the molecular level.

Findings: This year, we reported and published extensive molecular results from five long-term study sites (Hillside, Spray, Fairy, Witch, Western Pool). We also reported and published our implementation of this research project in our undergraduate teaching curriculum for molecular and microbiology coursework. In terms of new studies, we have three, all of which are in progress based on materials collected on this annual permit: 1) To address potential origins for red layer communities, we collected water samples from four long-term study sites and are in the process of analyzing 16S rRNA clones from sourcewater. 2) To visualize and demonstrate co-localization of retrieved DNA sequences with bacteria in the mat, we collected and fixed mat specimens for current in situ labeling studies, all of which are in progress. 3) To expand our ecological survey for new red layer communities, we collected new mat samples from two sites at Joseph's Coat thermal basin during a comprehensive GIS-based survey of this fascinating region. These samples have yielded informative bacterial sequences and we anticipate reporting and publishing this data alongside general GIS survey information in the upcoming year. All information has been or will be archived on our "Red Layer Microbial Observatory Database."

Project title: Analysis of Metal Resistance in Yellowstone Bacteria

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Additional investigator: David Barrie Johnson, Mark Delwiche, Heather Silverman,
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Objective: Isolation and characterization of thermophilic, thermoacidophilic, and other bacteria

and archaea from locations throughout Yellowstone to identify these microorganisms on the basis of 16S rDNA sequences and determine mechanisms of resistance/tolerance at the genetic level. Isolates may also be used in other INEEL research (V. Thompson, W. Apel, F. Roberto, co-PIs) screening for novel enzymes.

Findings: Three stable enrichment cultures have been obtained from 2001 sampling activities in and around Norris Geyser Basin and Crater Hills. Pools from which these enrichments (maintained at 700°C and an initial pH of 2.0) had measured temperatures of 850°C and pH 1.5-2. GPS coordinates and photodocumentation of these sites in August, 2001, are also available for these sampling locations. Initial characterization of microorganisms present based on 16S rDNA sequencing indicates these organisms to be members of the genus *Acidianus*. Collaborative work with other researchers is examining the distribution of archaeal viruses associated with these microorganisms (at least nine different morphologies have been observed to date). Studies of the microorganisms and associated viruses are ongoing.

Project title: Characterization of the Microbial Rhizosphere Population of Acid and Thermotolerant Grasses Associated with Hot Springs and Microbial Diversity in Thermal Soils in YNP

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Additional investigators: Lina Botero, Tracy Norris, Mark Burr, Jesse Donohoe-Christiansen

Objective: To study the diversity and identification of the thermophilic and acidophilic organisms associated with thermophilic plants located in YNP. We are also very interested in examining the diversity of the microbial community that thrives in select thermal soil locations.

Findings: We have obtained molecular evidence that some thermal soils (Temp. = 650°C to 850°C) apparently have diverse and complex prokaryotic communities. This study is continuing as we are developing new culturing techniques to cultivate maximal numbers of different prokaryotes from these soils.

Physiological and biochemical characterization of these different isolates is on going. Minimal soil disturbance has occurred; typically we use 1–5 gram of soil. We have discovered molecular evidence of extremely thermophilic *Pseudomonas putida* and *P. synxantha* (RNA-based RTPCR clones), and we have been able to cultivate these pseudomonads as part of a biofilm community. We have isolated a new extremely thermophilic bacterium having closest phylogenetic affiliation with a cluster of environmental PCR clones within the green non-sulfur division. Additional rRNA gene-based molecular work has uncovered RTPCR clones of Archaea that are only distant matches (79–81% sequence identity) to other Archaea sequences.

Project title: Microbial Biotransformations and Ecology

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Additional investigator: Andrew Laursen, Sophia Dore

Objective: To isolate microorganisms with unique metabolic activities allowing for the transformation of C-1 compounds, polycyclic aromatic compounds, and related products from the petroleum industry.

Findings: More than thirty strains of bacteria were isolated from thermal features in Yellowstone National Park that are capable of growing on C-1 compounds. We are currently conducting experiments with two isolates that are capable of growth at temperatures as high as 550°C, using methane as the sole carbon source. We believe these isolates possess the soluble form of the enzyme methane monooxygenase and could be useful in biocatalytic production of methanol at high temperatures. We are concluding experiments to further characterize these organisms based on internal membrane structure, 16S rDNA sequence, and functional gene analysis.

We have also characterized microbial structure in thermal features sampled during August 2000. DNA extracted from these samples was amplified by the polymerase chain reaction. We used denaturing gradient gel electrophoresis (DGGE) to separate DNA fragments based on nucleotide base sequence. In this way, we obtained a profile of genetic variability in these samples. We found that microbial diversity was greater in neutral to high pH thermal features than in acidic features, and there were changes in the composition of the microbial community related to pH. Surprisingly, diversity did not decrease at high temperatures, nor did the composition of the microbial community change as a function of temperature.

Project title: Effects of UV Radiation, Desiccation, and Heavy Metals on the Photosynthetic Microorganisms of Hot Springs and Associated Sediments

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Additional investigators: Tracy B. Norris, Timothy McDermott, Erich Fleming

Objective: The principal objectives that frame our proposed work are: A. Research concerned with *Cyanidium* and relatives. 1) Characterize *Cyanidium*/*Galdieria*/*Cyanidioschyzon* isolates to collect physiologic and phylogenetic criteria to unambiguously establish the genera and species that make up the *Cyanidium* complex and to distinguish them from other thermoacidophilic eukaryotic algae. 2) Determine if environmental features (e.g. heavy metals, pH, temperature, solar irradiance, competition, desiccation) may act as barriers to dispersion, or are correlated with specific *Cyanidium*/*Galdieria*/*Cyanidioschyzon* ecotypes within the various thermoacidic habitats.

B. Research concerned with cyanobacterial crusts near hot springs: Assess the contribution of scytonemin to the survival of cyanobacteria under desiccated conditions.

Findings: A. The work with *Cyanidium* and related eukaryotic algae involved the collection of live specimens from numerous thermoacidic habitats over much of Yellowstone National Park. Isolations of many strains were accomplished, and the ribosomal 18S DNA was sequenced for some of these, and at this point the nearest relatives of a few strains are closest to two species of *Galdieria*. Preliminary studies have shown that heterotrophic growth of most of the strains is negligible; some are unable to grow with nitrate as the nitrogen source; and growth on soil and water taken from various native sources containing heavy metals was slow to nil, but differed among strains (work done at Montana State University and University of Oregon).

B. The work with cyanobacterial crusts is in a very preliminary stage, and most of the work involves experiments with culture isolates at the University of Oregon.

Project title: The Biogeochemistry of Sublacustrine Geothermal Vents in Yellowstone Lake, WY

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Additional investigators: Carmen Aguilar, Robert Paddock, Russell Cuhel, James Maki, Charles Wimpee, Patrick Anderson

Objective: Principal Hypotheses: 1) Yellowstone vent throat habitats are capable of sustaining thermophilic microbial communities in situ, resulting in gradients of thermophilic to extreme thermophilic bacteria with different nutritional characteristics within vent conduits, from deep in the conduit to the mouth of the vent. 2) Vents in different basins will support different types of extemo-

phile communities because each biogeochemical domain provides vent water sources of differing composition with respect to energy sources (e.g., reduced iron, manganese and sulfur; hydrogen; methane). 3) Significant microbial activity occurs in vent field sediments, resulting from seepage of vent fluids and fracture zones.

Findings: Mineral inputs to Yellowstone Lake, WY come from a variety of sources, including hydrothermal vents, ground water, rainwater, flux from sediments and direct runoff. One third of Yellowstone Lake is directly influenced by hydrothermal activity (hot water vents and fumaroles). Geothermally heated water percolating through the chamber is highly enriched in carbonate, silicate, chloride, and methane, with some locations additionally rich in iron and sulfide.

Microorganisms that live in high temperature ecosystems are tightly coupled to their environment. A detailed understanding of the geochemistry of hydrothermal environments can be an important component in deciphering critical characteristics for the presence of microbial life under these changing conditions.

More than 25 chemosynthesis incubations included more than 20 vent samples and an array of associated water column samples. Due to weather and scheduling constraints, the West Thumb and Mary Bay areas of the lake were intensely sampled while Stevenson Island and outlying areas near Mary Bay were postponed until 2002. This permitted us to apply more detailed analyses of chemosynthesis in the two regions than had been possible in previous years. Especially improved in 2001 (but not yet optimized) was examination of high temperature chemosynthesis (50–700°C) in parallel with in situ temperature incubations.

Vent waters in West Thumb typically contained sub-micromolar concentrations of Fe while those in Mary Bay and off Stevenson Island contain up to 10M. The water column concentrations of dissolved Fe range from 250–450 nM in Mary Bay, but were below detection (180 nM) in the waters of South East Arm, West Thumb, and off Stevenson Island.

Pore water and vent water chemistry provide evidence for lake water dilution of vents below the sediment-water interface. Significant fracturing of source water conduits was indicated by extreme differences in pore water profiles from cores less than five m apart in geothermally vigorous West Thumb. Some samples approached theoretical reservoir composition for conservative geochemical tracers.

Porewater results from the geothermally active areas of Mary Bay and West Thumb show Cl-enrichments reaching several mmolar and, in the case of Mary Bay, extrapolate to the geothermal end member (~ 20 mM) at a depth of only 2–3 m. These steep concentration gradients support diffusive Cl- fluxes across the sediment-water interface three orders of magnitude higher than those in non-venting depositional areas.

Project title: A Survey of *Pilobolus* from Yellowstone National Park

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Objective: The objectives for the "A Survey of *Pilobolus* from Yellowstone National Park" are: 1) To determine what species of *Pilobolus* can be isolated in Yellowstone National Park. 2) To compare the various isolates for differing morphological characteristics of the isolates found. 3) To determine if the taxa from Yellowstone are the same as those found in different geographic areas. 4) To compare isolates of various taxa from various locations by contrasting morphological characters to DNA sequences and short chain fatty acid composition.

Findings: During 2001 isolates of *Pilobolus* were collected in Yellowstone National Park during May and August. These isolates were collected from mule deer, and elk from areas near Slough Creek, south of Solfatara, Roaring Mountain, the plateau north of Mammoth Hot Springs and about two miles south of Mammoth Hot Springs. All isolates maintained in the laboratory at Indiana University East are being used as part of larger studies to distinguish among the species of *Pilobolus*. Substantial progress was made in the past year using RFLP fingerprinting and DNA sequencing to resolve evolutionary relationships in *Pilobolus*. Two graduate student projects are in progress describing these techniques and the relationships found. It is hoped that these projects will be completed within the next couple of years. New fluorescent techniques have been attempted to show areas of growth in *Pilobolus* from Yellowstone. Results have been presented in Indiana Academy of Science meetings each of the past two years.

Project title: Bacterial Diversity of Thermophilic Anoxygenic Phototrophs

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Objective: The major objective of this research is to identify and culture photosynthetic microorganisms (anoxygenic photosynthetic bacteria) from Yellowstone thermal springs. In this regard, I am most interested in species of purple bacteria, green bacteria, and heliobacteria.

Findings: Several thermophilic photosynthetic bacteria have been isolated by my laboratory from Yellowstone thermal springs, most notably, the purple bacterium *Thermochromatium tepidum*, and the heliobacterium, *Heliobacterium modesticaldum*. All organisms isolated have been deposited in the American Type Culture Collection (ATCC, Manassas, VA), and are available for distribution to any qualified microbiologist. We continue enrichment and isolation experiments for new species, in particular, acidophilic photosynthetic bacteria.

No sampling was done in 2001 because we were scheduled to leave on September 12, 2001 for four days in Yellowstone and had to cancel due to the travel ban surrounding the terrorist attacks.

**Project title: Geochemical Constraints on the Ecology of the
Deep Lineages within the Bacteria and Archaea**

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Objective: 1) To determine the microbial diversity and geochemistry associated with high temperature thermal springs in YNP. 2) Study the ecology of microbial communities inhabiting YNP thermal springs.

Findings: Our research in 2001 was focused on Calcite Springs. We collected geochemical and molecular biological samples along chemical and physical transects in the springs. Additionally, enrichment culture techniques were used to isolate novel thermophilic microorganisms, including the bacteria that form black filaments in many of the near-neutral springs in Yellowstone. Our research in 2001 will focus on linking geochemical and community differences and using our cultures to understand the physiological diversity of Calcite Springs.

**Project title: Characterizing DNA Methylase and Restriction Enzyme Genes in
Environmental DNA**

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Additional investigators: Rick Morgan, David Ward

Objective: Restriction enzymes are one of the key reagents used in molecular biology. They recognize specific sequences within DNA and cleave at or close to this recognition sequence. More than 3,300 of these enzymes have been characterized worldwide, and more than 500 are available com-

mercially (Roberts and Macelis 2000). At present, we know of restriction enzymes that are able to recognize 240 different specific sequences. However, there continues to be an interest in finding both new specificities and new enzymes with specific properties that may make them especially suitable for certain applications.

We are interested in several outcomes from this research. First and foremost, we hope to isolate and characterize some new restriction endonucleases with novel properties, either ones that recognize completely new DNA sequences or far more stable isoschizomers of known enzymes that might be useful for specific applications where heat stability would be essential. Second, we hope to isolate and characterize DNA methylase genes, which comprise the second essential part of the restriction-modification systems we wish to study. We also aim to identify the organisms present in the samples studied by amplifying and sequencing the 16s rRNA genes present in the samples. To access the restriction-modification systems we isolate DNA from a thermophilic mat or filament sample, then prepare a DNA library from this DNA. We then employ one of several methods to identify clones that might carry restriction-modification genes.

Findings: One visit to Yellowstone was made in October of 2001, when, as the snow began to fly, samples of cyanobacterial mat, pool sediment and prokaryote filaments in run off channels were collected in the White Creek drainage area. The samples ranged from 500°C to 870°C and from pH 6.5 to pH 9. We are currently preparing DNA from the samples by a variety of methods, including bead-beating and chemical lysis. Construction of DNA libraries from the sample DNA is underway. The libraries will then be used for identifying restriction-modification systems. We have characterized a small number of 16s rRNA clones from Mushroom Spring, including several that are most similar (98% identity) to *Synechococcus* species isolated in Oregon by Miller and Castenholz (Appl Environ Microbiol 2000 Oct; 66[10]: 4222-9), several *Chloroflexae* type sequences and *Cytophagales* sequences. Characterization of more 16s sequence clones is ongoing.

Project title: Protein Comparison of Thermophiles and Oral Bacteria

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Objective: Oral bacterial microflora are extremely diverse (more than 300 different species in the normal oral cavity) and have to survive relatively large temperature and nutritional variations. Thermophilic microorganisms have been fairly well described, but no comparison has been reported with oral bacteria. It is proposed here to compare protein antigens between thermophilic and oral bacteria by immunological and electrophoretic (protein size) techniques. SDS-PAGE electrophoresis will be used to compare the sizes of proteins between representative thermophiles and laboratory strains of oral streptococci (primarily *Streptococcus mutans*, the causative agent of human dental caries). Immunological assays such as ELISA and western blots will be used to compare reactivity between antibodies to protein antigens on *S. mutans* and the thermophiles. It is anticipated that similar proteins will be observed between thermophiles and oral bacteria implying a possible

common ancestry.

Findings: Bacterial colonies were isolated on both selective and non-selective petri plates. Selected colonies were propagated and stored frozen until assayed. Samples were collected from human volunteers to compare to Park samples. Preliminary analysis of SDS-PAGE gel results indicates few proteins of similar size between the oral *Streptococcus mutans* and the Yellowstone microorganisms.

Project title: Ecology of Hot Spring Microbial Communities

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Objective: We have continued our long-term efforts to study the diversity, ecology, evolution and physiology of microorganisms inhabiting hot spring microbial mats. In essence, we treat the microbial mats of alkaline siliceous hot springs as models in which to investigate fundamental questions about the composition, structure and function of microbial communities. We also compare mats found in these and sulfidic hot springs, which are modern analogs of stromatolites, the most abundant fossils of the Precambrian Era, in an effort to study the present to learn about the past. Our specific objectives are as follows: 1) To evaluate the hypothesis that adaptive radiation of ecologically distinct populations explains the diversity of phototrophic microorganisms detected in mats of alkaline siliceous springs (e.g., Octopus Spring, Mushroom Spring) by analysis of sequence variation at the small subunit RNA (SSU rRNA) and 16S-23S rRNA intervening transcribed spacer (ITS) loci. To this end we are currently exploring the two-dimensional distribution of cyanobacterial genetic variants (i.e., with Department in mats at various temperature sites along the thermal gradient. This is being done in collaboration with colleagues at the University of Copenhagen Marine Biology Laboratory, who use microsensors to characterize the physical/chemical microenvironments and distribution of microbial activities (e.g. oxygenic photosynthesis) within the mats. We are also cultivating unicellular cyanobacteria (*Synechococcus spp.*) and characterizing their SSU and ITS genotypes to verify their relevance to in situ populations. Relevant isolates will be used in studies of phenotypic adaptation to temperature and other parameters that vary in the vertical aspect of the mat.

2) To evaluate the hypothesis that geographic isolation is involved in the diversification of hot spring cyanobacteria. To this end we have completed a study of the distribution of SSU rRNA and ITS genetic variants of hot spring cyanobacteria from local (e.g., within Yellowstone) to global (e.g., North America, Japan, New Zealand and Italy) scales, lineage-specific oligonucleotide hybridization probing to quantify the importance of genetically distinct types of cyanobacteria in different places,

lineage-specific polymerase chain reaction (PCR) to test distribution of putative rare genotypes, and chemical variation among springs.

3) To examine the diversity and distribution of green nonsulfur-like bacteria in mats of alkaline siliceous hot springs. To this end we have examined variation in SSU rRNA sequences at different temperatures in the Mushroom Sp. Mat, and developed and used fluorescent in situ oligonucleotide hybridization (FISH) probes to study the morphology, Departmental distribution and physiological activities of *Chloroflexus* spp. and their as yet uncultivated relatives in this mat.

4) To examine the diversity of green sulfur-like bacteria in sulfidic hot spring microbial mats. This has been done in conjunction with Dr. Donna Bedard (RPI), who will report results separately.

5) To study the organic geochemistry of phototrophic microorganisms inhabiting sulfidic and nonsulfidic mats in Yellowstone. To this end we have collaborated with colleagues at the Netherlands Institute for Sea Research (NIOZ) to study the lipid biomarkers of *Roseiflexus castenholzii*, a filamentous phototrophic bacterium genetically related to the uncultivated type-C *Chloroflexus* relatives inhabiting Yellowstone alkaline siliceous mats. We have also investigated the production, consumption and stable carbon isotopic signatures of lipids and carbohydrate biomarkers in unicellular cyanobacteria and filamentous green nonsulfur-like bacteria inhabiting mats of alkaline siliceous (e.g., Octopus Sp., Mushroom Sp.) or sulfidic hot springs (e.g., several in the Mammoth Upper Terraces Group).

Findings: 1) At all sites *Synechococcus* populations were yellow-green and chlorophyll-poor at the surface, but dark green and chlorophyll-rich in deeper layers. At 55 and 60°C, different *Synechococcus* SSU rRNA-defined populations were observed at different Departments. At 65 and 70°C no variation in SSU rRNA-defined *Synechococcus* populations was observed. Separate clades for surface or deep-layer ITS variants suggested that *Synechococcus* populations differently adapted to light inhabit different mat layers. At 65°C both surface and subsurface clones exhibited identical ITS sequences, suggesting either a single genotype acclimated to different light conditions, or distinct genotypes that are extremely closely related.

2) We have cultivated representatives of *Synechococcus* type A and B' SSU rRNA and ITS genotypes identical to those in situ. Additional ITS genotypes of these SSU rRNA genotypes, heretofore not found in situ, have also been observed, as have isolates closely related, but not identical, at the SSU rRNA locus. Type-B SSU rRNA genotypes were also recovered, but their ITS sequences are unlike those observed in situ.

3) Different predominant and diversified *Synechococcus* lineages occur in different countries. Type-A/B appear endemic to North America. They have undergone extensive evolutionary radiation, apparently due to both geographic and adaptive events. Type-C1 are predominant and diversified in Japan, but also rarely observed without genetic variation at low levels in a few North American springs. Type-C9 are in greatest abundance in New Zealand, where filamentous *Oscillatoria amphigranulata*-like cyanobacteria predominate. Although the filamentous cyanobacteria have diversified, unicellular cyanobacteria have not. Type-C9 *Synechococcus* were also in low abundance in North America and Japan, where their diversification is limited. Distribution patterns, combined with the lack of correlation with physical/chemical parameters, suggest that geographic isolation is important to diversification of hot spring cyanobacteria, though members of different lineages show different propensities for dispersal and colonization. The patterning of ITS sequence variation suggests that geographic isolation may be significant within Japan and the Greater Yellowstone Ecosystem.

4) We found a large diversity of *Chloroflexus* and type-C related SSU rRNA sequences. Both *Chloroflexus* spp. and type-C SSU rRNA sequences are associated with filamentous microorganisms. At 60° C, nearly all filaments were type-C organisms distributed throughout the photic zone. At 700°C, *Chloroflexus* and type-C each comprised about half of the filament population, the former restricted to the upper one mm. Type-C cells were observed to incorporate ¹⁴C-acetate, but not ¹⁴CO₂ during light incubation.

5) The main lipids of *R. castenholzii* were alkane-1, 2-diol glycosides or fatty glycosides and C37-40 wax esters. *R. castenholzii*-like organisms could be the source of structurally similar compounds found in mats, though in mats glycosides are less abundant and wax esters are shorter. We are investigating the possibility that carbohydrate biosynthesis in cyanobacteria could impart a heavy isotopic signature that is transferred to GNSB. We conducted a diel study of polyhexose variation in the Mushroom Sp. mat and are in the process of determining the stable isotope signatures of polymerized sugars in each type of organism.

Project title: An Analysis of Soil Microbial Community Structure in an Evolving Thermal Soil Environment.

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Objective: The objective of this work is to use molecular methods to analyze soil microbial community succession in response to changes in soil temperature. Investigations of the biology of hydrothermal systems have added greatly to our understanding of microbial species diversity and their evolutionary relationships. However, previous studies have generally been limited to thermal systems that are well established on the time scale of human observation. The death of lodgepole pines in this study site are indicative of a very recent expansion of the underlying geothermal plumbing. In some places temperatures as high as 80°C were recorded, which only six months previously were closer to 25°C. This study site provides us with a unique opportunity to observe changes in microbial community structure as they occur. This work will allow us to address questions concerning the forces affecting microbial community structure, diversity and the colonization of geothermal features by thermophilic microorganisms.

Findings: In this study, microbial species diversity was assessed across a landscape in Yellowstone National Park where an abrupt increase in soil temperature had occurred due to recent geothermal activity. Soil temperatures were measured and samples were taken across a temperature gradient (300–650°C) that spanned geothermally disturbed and unimpacted soils; thermally perturbed soils were visually apparent by the occurrence of dead/dying lodgepole pine trees. Changes in soil microbial diversity across the temperature gradient were qualitatively assessed based on 16S rRNA

sequence variation as detected with denaturing gradient gel electrophoresis (DGGE) using both rDNA and rRNA as PCR templates, and primers specific for Bacteria or Archaea. The impact of the major heating disturbance was apparent in that DGGE profiles from heated soils appeared less complex than the unaffected soils. Phylogenetic analysis of a bacterial 16S rDNA clone library PCR cloned from a recently heated soil showed that a majority of the clones belonged to the *Acidobacterium* (51%) and *Planctomyces* (18%) divisions. Agar plate counts of soil suspensions cultured on dilute yeast extract and R2A agar media incubated at 250°C or 500°C revealed that thermophile populations were 2–3 orders of magnitude greater in the recently heated soil. A soil microcosm laboratory experiment simulated the geothermal heating event. As determined with both RNA- and DNA-based PCR coupled with DGGE, changes in community structure (marked change in DGGE profile) of soils incubated at 500°C occurred within one week and appeared to stabilize after three weeks. The results of our molecular and culture data suggest that thermophiles or thermotolerant species are randomly distributed in this area within Yellowstone National Park and that localized thermal activity selects for them.

Project title: Viral Populations in Thermal Environments

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Objective: High temperature thermal environments (>75°C) are unusual among natural environments in their limited number of biological components and their relatively stable chemistries, temperatures and nutrient contents. The biotopes of thermal environments are comprised of the Bacterial and Archaeal cells and their cognate viruses. Few natural environments are so purely prokaryotic. It is becoming increasingly clear that viruses play a key role in the ecology of most, if not all, environments, presumably including thermal environments. While some research has been reported on thermophilic microbial populations, very little was known at the outset of this project regarding viral populations. One goal of this project is understanding the abundance and diversity of viral populations in these environments. A second goal of this work is to analyze the viruses to determine their utility for various applications.

Findings: Fifteen thermal features were examined (75–90°C, pH 4–9) for viral populations. Identity and diversity of viruses were examined by electron microscopy, environmental abundances were determined by epifluorescence microscopy and the biodiversity of their hosts was established by rDNA sequencing. For several of the thermal environments, a picture of the ecology is emerging that includes the phage diversity based on morphotypes, phage and microbial abundances based on epifluorescence microscopy, microbial diversity based on rDNA analysis. Molecular analysis of phage populations has begun.

Project title: Enhanced Practical Mitigation of Carbon Dioxide

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Objective: To isolate and characterize cyanobacteria from Black Sand Pools, Rabbit Creek and Mammoth Terraces that can be used in high temperature bioreactors (55°C) for the remediation of carbon dioxide from coal-fired power plants. The remediation equipment has been designed to allow its retro-fitting to the power plants. The cyanobacterial isolates have to be thermotolerant.

Findings: We have approximately 130 isolates growing at 55°C in three types of media. Eleven are unialgal. We have found that inoculum size is critical when the growth medium is gassed with a mixture containing 5% carbon dioxide and pH control is necessary. Growth curves based on chlorophyll a determinations show generation times as low as 8–10 hours and as high as 48 hours, depending on conditions (light level, carbon dioxide enrichment). The cyanobacteria were sampled from natural substrates in the aquatic environments mentioned above, or more efficiently, by allowing in situ colonization of artificial substrata. These artificial surfaces are those that are expected to be used in the pilot plant bioreactors.

Project title: Production and Consumption of Trace Gases by YNP Microbial Communities

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Objective: In order to be able to better characterize a biological signal in the composition of trace gases which may be emitted to the atmosphere, we made preliminary measurements of trace gas production by Yellowstone National Park hot spring microbial communities. It is our goal to use this information to help develop our search strategies for life through the detection of biomarkers in the atmospheres of extrasolar planets. Additionally, in preliminary work to investigate cyanobacterial sources of hydrogen, we made measurements of nitrogen fixation (using the acetylene reduction assay) by communities of the cyanobacterium *Mastigocladus laminosus*. Hydrogen production is an inevitable consequence of nitrogen fixation.

Findings: In this, our first field season, we concentrated on measurements of methane and hydrogen. The measurements of organosulfur compounds, originally planned for the 2001 field season, will instead be made in 2002.

Preliminary measurements were made on the hydrogen contents of gas bubbles emanating from the source waters of several springs, in order to gauge the potential for H₂ to provide a source of chemical energy, or of reducing power, for microbes in these systems. Gas bubbles in Mammoth region springs were generally very low in H₂, with concentrations consistently below 5 ppm. Highest concentrations (500–700 ppm) were observed in the acidic source waters of Norris Geyser Basin. For measurements in the Octopus Spring/White Creek and Rabbit Creek areas, a wide range of concentrations were observed (3–319 ppm). For these last areas, springs that contained significant photosynthetic microbial communities often exhibited markedly lower concentrations of H₂ in the venting gas than springs that had no visible photosynthetic communities, possibly suggesting biological utilization of upwelling H₂.

Methane concentration measurements were made in these same areas, both in source gas bubbles, and in bubbles of gas produced (or trapped) by microbial communities present in the springs. Methane concentrations in source water bubbles in the Mammoth area averaged around 300 ppm. Methane concentrations in the Norris Geyser Basin source gas samples averaged around 500 ppm. Higher methane concentrations were found in the Octopus Spring/White Creek and Rabbit Creek areas (averaging approximately 3,000 ppm). Methane concentrations in bubbles entrapped by microbial mats in all of the areas were much lower than concentrations of methane in source water gas bubbles. Incubations of microbial mat communities (to determine rates of methane production by these communities) confirmed that methane production rates in these mats are very low.

We used acetylene reduction assays to test for nitrogen fixation activity in two *Mastigocladus* populations, one from White Creek in the Lower Geyser Basin and the other from the Mammoth Terrace area. Water samples from White Creek lacked both ammonium and nitrate, and filaments of *Mastigocladus* contained abundant heterocysts. In contrast, the Mammoth site had high concentrations of nitrate (ca. 30 micromolar), and *Mastigocladus* had not produced heterocysts. Short-term (3 h) acetylene reduction assays demonstrated high rates of nitrogen fixation in the White Creek population (1.7 ppm ethylene produced per microgram Chlorophyll per h) and a lack of activity in the Mammoth population.

Future work: Methane could be produced biologically in the deeper, anoxic subsurface regions by organisms that utilize H₂ as an energy source. Isotopic analysis of this upwelling methane, which can help to discriminate between this biological mechanism and a purely geochemical one, is proposed as future work for these sites.

Project title: Genetic Analysis of Brucella from Bison and the Generation of a PCR-based Diagnostic System for Epidemiological and Ecological Studies

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Objective: The objectives of this work are to: 1) determine the genetic complexity of *Brucella* isolates from a variety of animal hosts, 2) develop a high sensitivity PCR based diagnostic system to identify the presence of *Brucella* isolates, 3) develop a PCR based diagnostic system to track specific genotypes of the *Brucella* isolates, 4) develop a PCR based diagnostic system to discriminate live *Brucella* cells from dead cells.

In addition to the objectives listed above, studies will be performed to convert the diagnostic systems to field adaptable systems capable of simple and rapid data generation.

Findings: We have completed the genetic analysis of *Brucella* isolates from several animal hosts including bison, cattle, and elk. These data are currently being incorporated into a scientific manuscript that will be submitted in 2001. In addition, several PCR primer sets have been prepared that amplify products specifically from *Brucella abortus* isolates. Protocols have been developed for extracting *Brucella* cells from blood samples and detection using the PCR diagnostic system. The genetic analysis has indicated that the strain RB51 used for vaccine development may be genetically unstable. This raises concerns over the use of RB51 generated vaccine as this strain may have the potential for reversion to virulence. This year, a manuscript will be completed and submitted to a peer-reviewed scientific journal.

Project title: Processes Maintaining Archaeal Biodiversity in Geothermal Environments

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Objective: Analyze diversity of prokaryotes associated with acidic sulfurous hot springs.

Findings: DNA sequence analysis of a number of YNP isolates has been done by a collaborator (Rachel Whitaker, University of California, Berkeley) under the terms of a material transfer agreement. Preliminary analysis indicates some DNA sequence diversity within a species of *Sulfolobus* that correlates with geographical location within YNP and also with locations outside the Park.

**Project title: Research Experience for Undergraduates: Yellowstone National Park
Field Trip, Summer 2001**

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Objective: The Research Experience for Undergraduates program at the Center for Biofilm Engineering, Montana State University, recruits talented students in various science, math and engineering disciplines to spend 10 weeks in Bozeman conducting biofilm research, learning effective technical communication skills and debating ethical issues that arise in technical fields of work and study. Yellowstone National Park serves as the perfect location to debate the ethics of harvesting microorganisms from natural environments. The students spent two days in the park observing wild type biofilms and discussing current biofilm research being conducted in the park.

Findings: The trip to Yellowstone Park increased the students' appreciation for field research. Viewing biofilm in a natural environment demonstrated the complex ecology associated with a living biofilm better than any bench-top laboratory system. The students left Yellowstone with a better understanding of the issues surrounding research in a national park.

Project title: Transition Between Lithoautotrophy and Chemoheterotrophy in *Sulfolobus* species

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Objective: To determine the factors, which regulate the metabolic status of hyperthermophilic archaea and bacteria in situ. To investigate methods for recovery of viable cells and the existence of postexponential phase physiological states. To assess rates of evolutionary drift in target genes.

Findings: The effect of sample pH, sample concentration, and sample ultrafiltration was examined on the recovery of viable cells from geothermal sites at various locations in the park. Preliminary evolutionary rates of drift were calculated for several target genes.

Project title: Isolation and Characterization of Thermophilic Viruses from Yellowstone National Park

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Additional investigators: George Rice, Jamie Snyder, Debbie Willits, Sue Brumfeild,
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Objective: The purpose of this project is to isolate and characterize the viruses from the hyperthermophilic acidophile *Sulfolobus*. Our long term goal is to use viral analysis as a means to understand the host organism and the adaptations necessary to survive in high temperature and low pH environments. There have been no previous analyses of the viruses from *Sulfolobus* in Yellowstone. However, viruses have been previously isolated from *Sulfolobus* from Japan, Iceland, and Italy. In this study, we would like to study the diversity of *Sulfolobus* viruses from within the park, to compare viruses from Yellowstone to viruses from other parts of the world, and to find new viruses that have never been discovered before.

Findings: We are successful at culturing *Sulfolobus* from Yellowstone and isolating viruses from those cultures. We have isolated viruses that are morphologically identical to isolates from Japan, Iceland, and Italy. However, based on preliminary genetic analysis, they are different. We have also isolated viruses that seem to be completely novel. We are continuing to find viruses from *Sulfolobus* and analyze them for biochemical and evolutionary studies.

Project title: Ecological, Physiological, and Molecular Biological Studies of Fungi from Geothermal Soils and Thermotolerant Plants in Yellowstone National Park

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Additional investigators: Joan Henson, Regina Redman, Kathy Sheehan

Objective: The proposed research will provide information to increase our understanding of fungal survival in unique environments, the roles of fungi in ecosystem dynamics, and the temporal and spatial scales of the micro-habitats that fungi occupy. Specifically, this work will provide information about: 1) how fungi survive under environmental conditions too harsh for mycelial growth; 2) if fungal community structure changes in response to environmental conditions; 3) if fungi can alter between saprophytic and symbiotic lifestyles in response to environmental conditions; 4) the scale of soil studies necessary to accurately assess the roles of these fungi in ecosystem dynamics; 5) how biological and/or genetic diversity of fungal communities changes in response to environmen-

tal conditions; 6) the adaptive mechanisms of tolerance required for the growth of fungi soils containing high levels of metals and other inorganic chemicals.

In addition, the feasibility of developing molecular biological tools will be determined for rapidly assessing a) fungal community structure based on molecular biomass measurements; b) the metabolically active, and inactive, species of fungal communities; and c) the occurrence of fungi in thermotolerant plants.

Findings: Several fungal species have been isolated and found to be either mesophilic or thermophilic. The populations of both fluctuate throughout the year as a result of soil temperature and moisture. Fungi are in highest densities in soil under plants and can be found in soils with temperatures up to 100°C. There are two classes of fungi present in the soils: saprophytic and symbiotic. Most of the fungi are very tolerant to heavy metals. This year extensive analyses will be performed to define the molecular genetic and ecological bases of the symbiotic interactions between these fungi and plants in geothermal soils.

Project title: Diversity of Thermophilic Anaerobic Microorganisms

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Additional investigators: Vadim Kevbrin, Chris Romanek

Objective: Elucidation of the biogeology, ecology, phylogeny, physiology, biogeochemistry, and diversity of thermophilic microorganisms. Presently with a special focus on alkalithermophiles as a novel group of thermophiles.

Findings: We had a very successful workshop meeting in Yellowstone National Park on the sister park in Kamchatka and how to preserve the Uzon Valley and Geysir Park in Kamchatka. The workshop included the visit of several of the hot spring areas. I collected some samples from the area behind Octopus Spring for measurements of alkalinity and isolations of alkalithermophiles growing above pH (60°C) 10. However we were not able to get anything new out of those samples.

Project title: Bacteria Living at Low pH and High Temperature

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Objective: Isolation and characterization of unknown thermophilic acidophilic microbes.

Findings: *Sulfolobus*-like microbes were sampled at 880°C and pH 2.2 at a site in Crater Hills, Great Sulfur Spring.

Project title: Phylogenetic Analysis of High-Temperature Ecosystems

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Objective: Ongoing research continues to focus on the survey of microorganisms in Yellowstone microbial ecosystems with varying solution chemistries. We have been measuring hydrogen concentration in the bulk aqueous phase to determine the variability in H₂ concentration in the Park's hotsprings. Small samples of biomass (1–10 grams) are obtained, and brought back to the lab for analysis by a molecular approach, rather than the traditional methodology of culturing. The molecular approach is based on cloning and sequencing of the small sub-unit ribosomal gene (16S rRNA gene) to determine the microbial composition of these ecosystems. Ongoing studies include analyses of sub-aqueous and sub-aerial systems for bacterial, archaeal, and eucaryal life.

Findings: Work from 1999 and 2000 on Well Y-7 in Biscuit Basin, has been written up and accepted for publication in *Yellowstone Science* for April 2002. We found that the sub-surface of Biscuit Basin has a varying temperature of its hot waters over the course of a year. We also found that the Well is rather devoid of life along its 250 foot length and that it has a thermal gradient of 50°C at the surface to 135°C at the bottom.

In 2001 we measured the bulk aqueous phase hydrogen concentration at a number of hot springs in the Park. We found high nM concentrations of H₂ at a number of locations, indicating that hydrogen, rather than sulfur, probably drives primary productivity in this geothermal ecosystem. This is supported by the molecular microbial studies done within the Park, where the overwhelming number of organisms utilize H₂ as the basis for their metabolisms. A manuscript on this work is in preparation.

Work from 1998 and 1999 on the formation of geyserite within the Park and its associated

microbiota has been completed. We see again, an extreme amount of microbial diversity, and a dependence on hydrogen. This work, with lead author Carrine Blank, has been written up and is about to be submitted to *Applied and Environmental Microbiology*.

Project title: Survey of Yellowstone Hot Springs for Green Sulfur Bacteria

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Objective: 1) To survey selected hot springs in Yellowstone National Park for the presence of thermophilic green sulfur bacteria (GSB). 2) To characterize thermophilic GSB found in Yellowstone hot springs by molecular techniques. 3) To further characterize and possibly isolate organisms whose 16S ribosomal gene sequences indicate that they may be deeply branching relatives of green sulfur bacteria from selected Yellowstone hot springs.

Findings: We have continued to follow up on the GSB located in several hot springs in Yellowstone National Park in 2000. This year we revisited sites in the Mammoth Hot Springs region, Gibbon Hills area, and the Mud Volcano region. We confirmed that the GSB found last year in the latter two sites are still present although the temperatures and appearance of the sites have changed. We have not been able to demonstrate the presence of any GSB in the Mammoth Hot Springs area. We have sequenced an 800 bp region of 16S rRNA genes from GSB enrichments and from DNA extracted from hot spring mats and amplified with GSB-specific primers. We have confirmed that sequences from GSB found in two different regions, the Gibbons Hills area and the Mud Volcano region, are distinct from those of the thermophilic GSB *Chlorobium tepidum* previously identified in New Zealand. We are continuing our analyses.

ORNITHOLOGY

Project title: Aspen–Migratory Bird Relationships in the Northern Yellowstone Ecosystem

Principal investigator: Dr. William J. Ripple
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Additional investigators: Jeff Hollenbeck

Objective: Our objective is to determine the relationships of aspen patch characteristics and stand composition with migratory and resident birds. Previous research at lower elevations in the western United States suggests that migratory birds may respond to patch level attributes of aspen stands when settling on their breeding range. Specific objectives include determining whether the relationships found between patch characteristics and breeding bird abundance at lower elevations occur more widely and the effect of conifer invasion of aspen stands on bird diversity. We measured several patch-level and within-patch characteristics of 30 aspen patches throughout the northern ungulate range and surveyed birds using point counts.

Findings: The first of two field seasons yielded 1,129 observations of 46 species of birds, the majority of which are Passerine migrants. The basal areas of aspen and conifer, canopy covers of aspen and conifer, dominant understory vegetation, area, perimeter, elevation, azimuthal orientation of major axis, and matrix context of each aspen patch were measured. Analysis of relationships between patch characteristics and bird abundance and species richness is planned after the next (2002) field season.

Project title: Relating Avian and Herptilian Abundance and Diversity to Human Disturbance Regimes

Principal investigator: Mr. Eric Atkinson
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Additional investigators: Melonie L. Atkinson

Objective: 1) Identify areas of significant avian abundance and diversity within riparian zones on

the Northern Range. 2) Identify areas supporting amphibian breeding, foraging, and dispersal activities within riparian zones on the Northern Range. 3) Characterize above areas through habitat and environmental description at local and drainage scales in order to relate to patterns in avian species assemblages and herptile (amphibian) breeding and foraging sites. 4) Relate diversity and abundance patterns to anthropogenic disturbance regimes within and between drainages.

Findings: During our second year of field investigations we sampled 387 point count plots for birds yielding over 5000 individuals comprising over 100 species. Like year 2000, the most numerous passerines detected within 100 m on point counts were Cliff Swallows (*Petrochelidon pyrrhonata*) owing to their gregarious nature, American Robins (*Turdus migratorius*), and Savannah Sparrows (*Passerculus sandwichensis*). Savannah Sparrows were found in sedge (*Carex sp.*)-dominated areas whereas at the other end of the vegetation gradient, Lincoln's Sparrows (*Melospiza lincolnii*) were consistently found in willow (*Salix spp.*) stands. Warblers were absent from most sites exclusive of high elevation willow sites and those sites where willows were a major component of the vegetation. Notably, one male Harlequin Duck was encountered on our avian point counts.

Thus far, we have identified significant drainage effects upon levels of riparian bird community diversity and abundance. Bird abundance, species richness, and diversity were positively related to willow abundance but showed varying patterns between drainages on the Northern Range. Avian diversity at sites within YNP fall midway within the range exhibited by sites outside of YNP but within the Northern Range.

We are refining measures to more accurately describe species replacements associated with habitat features such as basin characteristics, floodplain size, and riparian shrub height and density. Development of an Index of Biotic Integrity (aka., Bird Integrity Index) relating specifically to shrub-dependent passerines is ongoing. Such methodology shows promise in characterizing the riparian 'health' of stream reaches that may be decoupled from aquatic chemistry profiles.

These same drainages have been surveyed for amphibians and reptiles identifying another previously unknown boreal toad (*Bufo boreas*) breeding site as well as breeding sites of Columbia spotted frogs (*Rana lutieventris*) and boreal chorus frogs (*Pseudacris maculata*). The boreal toad breeding site identified in 2000 was occupied and successfully produced metamorphs in 2001. Moreover, we identified areas that appeared to harbor young toads through the winter, as we encountered them as they moved along water bodies in early season.

Project title: Movements and Survival of Bald Eagles Banded in Yellowstone National Park

Principal investigator: Dr. Alan Harmata

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Fish and Wildlife Program

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Additional investigators: George Montopoli

Objective: Movements and survival of bald eagles produced in Yellowstone National Park, including nestling gender assignment methods and environmental contaminant loads in tissue.

Findings: Four nestling bald eagles banded in YNP in 2001, and blood samples for DDT analysis and DNA gender assignment obtained from two.

OTHER

Project title: Science and Scientific Research in Yellowstone National Park: An Internet-Searchable Bibliographic Database

Principal investigator: Ms. Cynthia Kaag
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Objective: To provide one central “catalog” of all accounts of scientific research, whether or not formally published, conducted in and around Yellowstone. Entries will include bibliographic information as available and annotations wherever possible.

Findings: The database (<http://www.wsulibs.wsu.edu/yellowstone/>) currently includes over 10,600 unique entries for articles, reports, books, studies etc. dealing with scientific research in Yellowstone and the Greater Yellowstone Area. During the past year ca. 400 new entries were added, and ca. 150 old entries were updated with corrected or additional information. This database is freely available to researchers worldwide and the investigator serves as an additional information resource, answering questions by email.

Project title: Animal–Vehicle Crash Mitigation Using Advanced Technologies

Principal investigator: Mr. Pat McGowen
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Bozeman, MT 59717

Additional investigators: Justin Farrell

Objective: The objective of this re-application is to seek compliance with research permitting in Yellowstone National Park (YNP) for the purposes of continuing the Animal–Vehicle Crash Mitigation pooled-fund study. Animal Vehicle crashes are a significant problem on the nation’s rural byways. Concover et al. (1995) estimates that collisions with large animals account for an estimated 726,000 collisions in the US annually. Traditional countermeasures have attempted to 1) limit animal presence on the roadway using fences, reflectors, scent and sound repellants, intercept feeding, or increased hunting; 2) improve the motorists ability to react through speed zones, roadside Clearzones, or highway lighting; or 3) improve the driver’s awareness of the hazard through warning signs and public education.

With the advent of the Intelligent Transportation System paradigm for transportation management and an increased focus on advance technology solutions, the Oregon Department of Transportation and the Western Transportation Institute (WTI) at Montana State University have re-examined the problem of animal–vehicle collisions. Twelve State Departments of Transportation have funded a study to develop, install, and evaluate a system built by Sensor Technologies and Systems, Inc. (STS). The system has been delivered and will be installed by the Montana Department of Transportation (MDT) in May–June 2002. The system will reside within the existing right-of-way. Specific objectives to be completed in 2002 include: 1) pilot roadside observation February–March 2002, 2) installation of the animal-detection, driver-warning system (MDT) May–June 2002, 3) de-bugging June–July 2002, and 4) evaluation Fall–Winter 2002.

The intent of this re-application, project, and evaluation is in keeping with the purpose, mission statement, and goals of Yellowstone National Park. This project will lend to the fostering of Goal Category IVa (current technologies) and Goal Category IVb (park partnerships) of the TNP Strategic Plan/Mission Statement.

Findings: While progress on the delivery and installation of the prototype system was stalled to Spring 2002, progress in the study was achieved. Past year activities have involved continued consensus building among study partners, including the addition of a new partner, the Alaska Department of Transportation. WTI is currently courting partners in Kansas, New Jersey, and Pennsylvania. The design of a prototype radar detection system was completed by STS in November 2001 and delivery completed shortly thereafter. The system is currently in storage with MDT in Bozeman and awaits deployment. WTI is currently working out some design bugs with STS and as such all fieldwork has been deferred until 2002. Objectives to be completed are as listed above. A second test location (as yet undetermined, but outside YNP) will demonstrate Oh Deer, Inc. technologies.

Project title: Ecology of Phototrophs in Extreme Environments: Thermal and High Iron

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Additional investigators: Brian Beard, Zack Stockdale

Objective: We are trying to determine the distribution and role of both oxygenic and anoxygenic phototrophic microorganisms in extreme environments. We are trying to determine how these bacteria interact with each other, with other microbes and with the physical/chemical environment. We are especially interested in their interactions with iron. Ultimately we hope to better understand the early evolution of photosynthesis and the impact of photosynthetic microbes on Earth in the Precambrian.

Findings: For personal reasons I was unable to spend much time at our sites in the park this summer. We were able to verify the presence of low oxygen levels in microbial mats in high iron hot springs compared to much higher oxygen levels in similar mats found in low iron hot springs. We also demonstrated a stimulation of photosynthesis by iron. Most of our measurements were done with microelectrodes in situ. Preliminary analysis of iron isotope composition of sediment and water samples from high iron springs showed variations in fractionation that could be due to changes in iron speciation, kinetic effects due to rapid precipitation, and/or biological oxidation.

Project title: Collection for Interpretive Educational Programs

Principal investigator: Ms. Judith Knuth Folts

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Objective: 1) Provide visitor education on cultural and natural history interpretive themes at Yellowstone National Park through the use of interpretive props including, but not limited to, rocks, antlers, bones, fur, feathers, etc. 2) Provide information on park critical resources and management to engender an understanding and appreciation of park significance.

Findings: During FY 2001, the Division of Interpretation completed 4,482 formal interpretive education programs, 120 curriculum-based environmental education programs, and 7,750 hours of roving interpretation. More than 382,000 visitors attended these environmental education programs, walks, talks, hikes, and evening campfire programs, or they talked with an Interpretive Park Ranger on rove assignment at campgrounds, pullouts, or wildlife jams. 2,040,899 visitors were contacted at the nine in-park visitor centers and four winter warming huts during the fiscal year.

All Interpretive Park Rangers used various field specimens as interpretive props during their park employment. These tangible aids assisted visitors in learning about the parks resources, protection policies, and visitor/wildlife interactions. Props are vital to a ranger's ability to relate a compelling story to visitors to engender a stewardship ethic about parks.

PALEOECOLOGY

Project title: Evolution and Ecology of Vertebrates of Yellowstone National Park—Continuation of Lamar Cave and Waterfall Locality Paleoecology Research

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Stanford, CA 94305-5020

Objective: Our objective was to determine whether we could map the origin of faunal and floral material through the past 3,000 years using strontium isotopic analyses.

Findings: One of the greatest challenges in using faunal assemblages to make ecological or paleoecological interpretations is determining the spatial scale over which such analyses are applicable. As a result, it has been difficult to use these assemblages to test hypotheses about spatial and temporal variability in populations. Here we show that it is possible to use strontium (Sr) isotopes from bones and vegetation to statistically constrain the area sampled in two Holocene predator accumulations in northeastern Yellowstone National Park, Wyoming. The sites have been used to elucidate local population responses to climatic change, based on the assumption that the specimens originated within ~5 km of the site. We used Sr analyses to construct a likelihood curve that describes the probability that our samples were collected within a given radius of each site. Our results indicate that the specimens in both sites were derived from non-overlapping populations and that the collection radius has not changed detectibly over the past 3,000 years. This work underscores the promise of this technique for ascribing source areas to paleontological, biological and ecological specimens.

PALEONTOLOGY

Project title: Paleontological Inventory and Multi-Disciplinary Assessment of Stratigraphy and Erosion Factors on Mount Everts, Yellowstone National Park

Principal investigator: Ms. Elaine Hale
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Additional investigators: Vincent Santucci, Scott Wing, William Cobban, Dr. Jason Hicks

Objective: The objectives of the two year project are to conduct systematic scientific survey of the rock formations and alluvial deposits within the fossil bearing region of Mt. Everts. The project will provide paleontological documentation, stratigraphic assessment, and baseline data necessary for management decisions concerning resource protection and infrastructure development.

Mt. Everts is located in the northern portion of YNP, bounded on the western edge by the North Entrance Road. The stratigraphy of Mt. Everts is not well understood and is known to contain Cenozoic and Mesozoic fossiliferous exposures. The Cretaceous strata on Mt. Everts display an interfingering of marine and terrestrial units. A variety of fossil types including aquatic and terrestrial plants, vertebrates, invertebrates, and trace fossils have been recovered from Mt. Everts.

Field survey is needed to provide baseline geologic data on fossil localities, stratigraphic data related to the geology of the localities, paleontological data related to the identification of paleotaxa present, and geologic data related to the depositional environment of the fossiliferous units.

Findings: Prior to fieldwork a literature search was conducted to identify all of the known research previously conducted in the Mt. Everts area. Previously identified fossil localities were revisited, stratigraphic interpretations synopsisized, and an attempt to locate fossils previously collected from Mt. Everts conducted. The Mt. Everts fossils in the YNP museum collection were accessed and re-examined. The known and questioned depositional characteristics of the area were synopsisized and combined with the literature list, fossil species list and stratigraphy charts.

An interdisciplinary team of experts representing a wide array of geologic and paleontologic expertise was assembled to participate in the project. Participants in the 2001 field investigations include Dr. William Cobban, Paleontologist Emeritus with the U.S.G.S. and Cretaceous index fossil expert; Dr. Scott Wing, Paleobotanist with the Smithsonian; Dr. Karen Porter, Senior Research Geologist with the Montana Bureau of Mines and Geology; Dr. Thaddeus Dyman, U.S.G.S. Paleontologist and stratigrapher; and Dr. Gail Wiggett, California Department of Energy, trace fossil and sediment analysis; and Vince Santucci, Paleontologist with the NPS.

Project title: A Continuing Investigation of the Eocene Palynoflora of the

Yancey Creek Drainage Basin, Yellowstone National Park, Wyoming

Principal investigator: Dr. Robert Jorstad
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Eastern Illinois University
Charleston, IL 61920

Additional investigators: Craig Chesner, Melissa Stefos

Objective: To gain an understanding to the paleoenvironmental and floristic nature of the Eocene Sepulcher Formation in northeast Yellowstone National Park.

Findings: EIU Geology Field Camp students assisted with the collection of ten samples from the Yancey Creek area near Lost Lake during July 2001. This site is approximately 2–3 kilometers from the summer 2000 sample site (Jorstad, Stefos, and Chesner 2001). This study does not evaluate the “in situ” or “transported” models associated with the fossil trees found near Specimen Ridge as discussed by Fritz, 1980.

The volcanoclastic rock samples, from the Eocene Sepulcher Formation, are being processed using standard acetolysis techniques for palynological study as described by Doher, 1980. The Sepulcher Formation consists of sedimentary interbeds between volcanic members of the Absaroka Supergroup. The Sepulcher deposits (53–49 my, Hiza, 2000) significantly pre-date the Huckleberry Ridge Tuff (2.0 my), Lava Creek Tuff (600 ky), and the Mesa Falls Tuff (1.3 my) associated with the major caldera forming events in the park.

Melissa Stefos has identified the following pollen in the samples from the Yancey Creek drainage basin. This floral list is developed from analysis of slides made from samples collected during the summer of 2000. The samples collected in 2001 are still being processed for study. Identifications have been based on comparison with published illustrations and descriptions using an Olympus microscope at over 400X.

Palynomorphs found in Eocene volcanoclastic sedimentary rocks from the Sepulcher Formation in Yellowstone National Park include: *Picea*, *Abies*, *Pinus*, *Spagnaceae*, *Nymphaea*, *Acer*, *Quercus*, *Genmcanadense*, *Carya*, *Celtis*, *Prunus*, *Chamaecyparis/Cupressus*, *Betula*, *Fagus*.

These findings are consistent with reports of other workers. Recently another student, Sarah Marosek, has gotten involved with the research program and has reported finding a grain of *Ostrya*. The identification work continues.

The total number of identified grains at this time is insufficient to make any reliable paleoclimatic or ecological interpretations. Initial impressions certainly suggest the presence of a mixed coniferous–deciduous forest during the Eocene.

RESTORATION

Project title: Yellowstone Gray Wolf Restoration Project

Principal investigator: Dr. Douglas Smith
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Objective: Monitoring reintroduced wolves in Yellowstone National Park.

Findings: 132 wolves in 10 packs in Yellowstone National Park; 216 wolves in 24 packs in the Greater Yellowstone Ecosystem (GYE); 14 breeding pairs in the GYE. About 77 pups survived. Average pack size = 13. Collared 32 wolves.

Project title: LeHardys Rapids Yellowstone Cutthroat Egg Collection for the Development of Species Specific Brood Stock for Drainage Restoration

Principal investigator: Mr. James Barner
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Additional investigators: Brian King, Pete Feck, Paul Krestchmar

Objective: To collect and fertilize eggs from Yellowstone Cutthroat trout (*Oncorhynchus clarki bouvieri*) pairs to develop a captured brood stock program. Traditionally eggs have been collected from the population that inhabits the Yellowstone River from Yellowstone Lake to the Upper Falls with the primary capture location being LeHardys Rapids. With the extremely low water levels of 2001 and difficulty capturing fish at LeHardys Rapids, fish that ran Clear Creek were captured, eggs were collected, and the progeny will be used for brood recruitment depending on genetic evaluation.

The original objective was to collect a partial spawn from a minimum of fifty pairs for three consecutive years (2001–2003) but due to a catastrophic flash flood which washed out the brood pond at the Clarks Fork facility spawning will need to be carried out for at least three more years to establish this stock. The intent of this stock is restoration of the endemic range of Yellowstone Cutthroat in Wyoming and Montana. If low water conditions persist we would like to utilize the Clear Creek population for recruitment again.

Findings: Eggs were taken on two occasions from June 21 to June 27, 2001. The collections were as follows: Lot Number = Year source species take, Year = Two digit year the fish were collected

(2001=01), Source = Spawning location code (Clear Creek = 57), Species = Three letter species code (Yellowstone Cutts = YSC), Take = Sequence the spawn was taken (1st spawn = 01), B1 = Indicates lot is brood recruitment, therefore only a partial spawn was removed from the female.

Lot 0157YSCB1: Spawned 01/06/21, females = 45, males = 45, eggs = 16650. Water temperature = 50°F, air temperature = 65°F, shipped to: Tillett Isolation Facility, Lovell, Wyoming.

Lot 0157YSCB2: Spawned 01/06/27, females = 37, males = 37, eggs = 13328. Water temperature = 48°F, air temperature = 55°F, shipped to: Tillett Isolation Facility, Lovell, Wyoming.

A total of 86 pairs were collected on the two dates above. A total of 86 ovarian samples were collected from the spawning population of Clear Creek. The green fertilized eggs were incubated at the Tillett Isolation Facility.

**Project title: The Economic Value Associated with Substitution Away from
Yellowstone National Park as a Unique Recreation Site**

Principal investigator: Dr. John Loomis

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Additional investigator: Chris Bastian

Objective: Specific objectives of this research are to: 1) Complete a survey of snowmobilers using Yellowstone National Park which addresses their substitution to another site subject to YNP being closed to snowmobile use; 2) estimate a random utility model (RUM) from the survey data that estimates probabilities of potential use of substitute sites and changes in recreation benefits associated with those substitutions; and 3) compare actual substitution to stated preference responses regarding estimated probabilities and benefits in a random utility model of recreation demand.

Findings: Intercept sampling of snowmobilers entering Yellowstone National Park between December 20, 2001 and February 28, 2002 was completed. The West, South and East entrances were sampled on 34 dates during the above time period. Dates were chosen randomly for both week and weekend days during the period. An equal number of week and weekend days were sampled during that period. The total number of days each entrance was sampled was based on proportion of snowmobile visitation during the 2000–2001 winter recreation season. A total of 1,280 visitors were intercepted and asked if they would participate in a survey regarding their snowmobile use. There was a refusal rate of 9.5% during the intercept sampling. Those agreeing to participate were entered into a database. A modified Dillman survey design was started with the first mailing on March 4, 2002, and a postcard follow-up reminder sent out on March 11, 2002. The third and final survey mailing will occur on April 1, 2002. As of the submission of this report 483 surveys out of 1,148 have been received. Data entry, analysis and the submission of our findings will occur by the end of November this year.

Project title: Visitor Experience and Media Effectiveness

Principal investigator: Dr. Robert Eisenberger

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Additional investigator: Ross Loomis

Objective: National parks preserve irreplaceable natural and cultural resources. These parks and resources provide educational, inspirational, and recreational experiences for tens of millions of visitors each year. Understanding how interpretive media (e.g., exhibits, audiovisual presentations, brochures, and maps) contribute to visitor satisfaction and resource protection will help the National Park Service improve the effectiveness of interpretive media and the quality of visitor experiences. The purpose of the proposed research is to develop a basic understanding of how visitors' differing needs and desires act in combination with media experiences to influence visitors' satisfaction, and practical methodologies that can be used in the future to design and evaluate media that will better achieve visitor experience and resource protection objectives.

Findings: Data collection has been completed. The data is currently being analyzed.

**Project title: From Juno to Toby: Policy, Culture, and the
Historical Narrative of the Yellowstone Bear**

Principal investigator: Dr. Timothy Oakes

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CB 260

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Additional investigator: Alice Wondrak Biel

Objective: 1) To determine the importance of seeing a bear to the overall quality of today's visitor experience, 2) to assess attitudes toward bear feeding, and 3) toward seeing research tools such as satellite collars and ear tags being worn by park wildlife.

Findings: 1) In response to an open-ended question, about half of all 150 visitors surveyed named bears as one of the top three sights they wanted to see in Yellowstone, second only to Old Faithful. Seeing a bear was ranked as being of only average importance to the overall quality of experience, however, indicating that visitors would like to see bears but are not excessively disappointed if they do not. 2) 95% of survey respondents claimed that they would not want to feed a Yellowstone bear. 3) 83% of visitors surveyed said that seeing a collared animal in Yellowstone either "had not" or "would not" affect their experience of viewing that animal. Some respondents actually expressed interest and/or pleasure at the idea because they supported research for conservation purposes (their reasoning). This seems to indicate that the long-held notion that allowing wildlife to be collared and otherwise marked for research purposes is disruptive to the visitor experience is based on incorrect assumptions about park visitors.

VOLCANOLOGY

Project title: Contemporary Surface Deformation of the Yellowstone Caldera

Principal investigator: Dr. Daniel Dzurisin

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Cascades Volcano Observatory

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Objective: Every few years, we will conduct precise leveling surveys along the roads of Yellowstone National Park to assess the character of vertical deformation within and around the caldera system. These surveys began in 1983 and will continue as long as funding is available. The next survey is likely in the summer of 2003.

Findings: No work completed during 2001 calendar year.

Project title: Monitoring Caldera Unrest at the Yellowstone Caldera: A Global Positioning System (GPS) Crustal Deformation Study and Hot Springs Temperature Study by the Eastern Illinois University Geology Field Camp

Principal investigator: Dr. Craig Chesner

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Eastern Illinois University

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Additional investigators: William Toothill, Sid Halsor, Robert Jorstad

Objective: The purpose of our study is to monitor caldera unrest at the Yellowstone Caldera by annually collecting GPS data and hot springs temperatures from a network of data collection points. Although the data collected in this study can contribute to more detailed monitoring efforts of the caldera, the primary goal of this project is to provide an ongoing, hands on field experience for undergraduate geology majors at the Eastern Illinois University Geology Field Camp.

Findings: During our third annual GPS survey on June 27–28, 2001, we deployed three Trimble 4000 SSI receivers at pre-determined GPS control stations in the park. These stations are located along a NW-SE traverse across the western part of the Yellowstone Caldera. Each year, data is col-

lected continuously for 48 hours from eight control stations. The GPS team sets up each station and trains two students in overall instrument monitoring before leaving them with the receivers for four-hour intervals.

A comparison between our 2000 and 2001 data indicates that uplift has resumed across the western region of the Yellowstone caldera. Uplift ranged between one and eight cm along our traverse, generally increasing northward. Subsidence ranging between 3 and 10 cm occurred across our network between 1999 and 2000. This shift from subsidence to uplift is consistent with the preliminary results reported by Robert Smith, University of Utah. However, the magnitude of our vertical deformation rates across the network is markedly higher than those reported by Smith.

The 2000 to 2001 horizontal displacements along our traverse ranged from a few mm's to three cm. Overall displacement direction was to north, varying between NNE and NNW. The largest horizontal displacements occurred at stations with the smallest uplift. Between 1999 and 2000, horizontal displacements were mostly westerly with a southern component. This shift to northerly movement contrasts with the southwesterly displacement defined by the regional deformation model across the Yellowstone Plateau. If our data are applied to this model, the axis of "rising and stretching" is located south or southwest of the present-day caldera. Our working group continues to examine the reliability of our results in view of the regional deformation patterns. A more detailed report of this study, including data tables and a map is available at: <http://146.94.14.10/yellowstone>.

A second component of our study is monitoring hot springs temperatures at selected thermal areas within the Yellowstone Caldera. Our study area consists of three separate thermal areas in the Lower Geyser Basin located along Rabbit Creek, White Creek, and Sentinel Meadows. Data is collected by small groups of students led by a faculty member who evaluates each thermal feature for safety and the best location to measure its temperature. Students measure the temperature of each hot spring using thermocouples and record the highest temperature obtained. The location of each measurement point is determined precisely in terms of latitude and longitude using a roving GPS unit carried by another student. Baseline data for our study was collected on June 28–29, 2000 when over 100 thermal springs were measured.

On June 27–28, 2001 we revisited the study areas and collected temperature and location data on 146 hot springs. Temperatures recorded at hot springs in Sentinel Meadows were essentially unchanged from 2000, measuring mostly in the 90's, with several as high as 94°C. At least three new pools have become active south of Sleep Cone north of the main trail. The consistently high temperatures qualify Sentinel Meadows as the overall hottest of our three study areas. Although most hot springs in the Rabbit Creek area remained unchanged, significant increases of 4–19°C occurred at multiple pools in three separate hot spring clusters. Thus, Rabbit Creek qualifies as an area with an overall temperature increase. Data collected from White Creek was more difficult to evaluate because many of the 2001 locations were new, or did not correlate with the 2000 locations. Hot springs that did correlate between the data sets were essentially unchanged. Rabbit Creek and White Creek thermal features were more variable than Sentinel Meadows, with temperatures ranging from about 38–94°C. Maps and data tables for this study can be viewed at: <http://oldsci.eiu.edu/geology/camp/YNP/ynpres.htm>.

Project title: Physical Volcanology of the Huckleberry Ridge Tuff

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New Zealand

Objective: The objective of the research is to collect detailed quantitative information on the internal physical and chemical stratigraphy of the Huckleberry Ridge Tuff, from representative sections within and beyond Yellowstone National Park. This information will be used to enhance understanding of the vent locations and their evolution, the timing and development of caldera collapse, the transport and emplacement of the ash flows, and the overall time-scales of the parental eruption.

Findings: A total of 13 field days were spent working in Yellowstone National Park, accessing easily-reached areas around Mammoth, Mount Everts, and the Blacktail Deer Plateau north to the Yellowstone River. Work within the Park forms part of a much broader study of the Huckleberry Ridge Tuff (HRT), and the work in 2001 was intended as a reconnaissance to acquaint myself with features of the tuff. This aim was accomplished, but in addition several new discoveries were made that extend the pre-existing understanding of the stratigraphy and distribution of the tuff. These are as follows: 1) A new basal locality with fall deposits preserved below the tuff has been found at a site north of Yellowstone River, about 400 m west of Little Cottonwood Creek. This and other sites at Mount Everts indicate the HRT eruption started with only-weak, 'sputtery' explosive activity before escalating into the large-scale ash-flow producing activity. 2) Study of the basal fall deposits exposed on Mount Everts has shown that ash flows were being emplaced nearby for some significant time period prior to the level represented by the onset of ash-flow deposition at the preserved sites. This implies that the onset of ash-flow deposition of HRT Member A is diachronous, and that earlier portions of the tuff were coeval with, not following, fall deposition. Thin surge and flow deposits found at the northern tip of Mount Everts thin southwards over 2–3 km to vitric ash fall, implying the coeval flows were emplaced nearby, in a now lost paleovalley through the Mammoth–Gardiner area. 3) New localities were found that exposed crystal-poor tuff that is equivalent to Christiansen's (2001) HRT Member C just west of Mammoth, specifically, on the southern slopes of Terrace Mountain and the ridge east of Clematis Creek. Although stratigraphically equivalent to Member C (i.e. overlying the visually distinctive top to Member B with a clear welding [hence cooling] discontinuity), this newly discovered material is different in appearance to the archetypal Member C material at Huckleberry Ridge and the Red Mountains (near South Entrance) and their genetic relationships require clarification. 4) Comparison of sections within and outside the park have shown that the distribution of indentifiable sub-units ('facies') within the HRT members is more complex than previously thought. For example, crystal-poorer parts of HRT Member A form only thin (dm to m-thick) basal and top parts of the member where exposed on Mount Everts, but appear to form the bulk of Member A where exposed in the Madison River valley west of the park.

I greatly appreciate the opportunity to undertake work in the park, and am encouraged by the findings to date that information from the park will be of importance in my overall study of the HRT. No material has been prepared for publication from 2001 efforts, due to the preliminary nature of the findings and the need for more detailed future sampling and documentation.

Project title: Eruption Observation of Selected Remote Geysers

Principal investigator: Mr. Jeff Cross
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Additional investigator: Carlton E. Cross, Tara M. Cross

Objective: To obtain eruption interval and duration measurements of selected remote geysers at Shoshone Geyser Basin (Double Geyser, Frill Spring), Heart Lake Geyser Basin (Glade Geyser), Lone Star Geyser Basin (Buried Geyser, Unnamed geyser SW of Lone Star-Shoshone trail bridge), Gibbon Geyser Basin (Phoenix Geyser).

Findings: Shoshone Geyser Basin: Double Geyser erupted every 4,300–5,643 seconds (mean of 35 intervals = 4,664 seconds); Frill Spring was dormant. Heart Lake Geyser Basin: Glade Geyser erupted every 2–5 days. Gibbon Geyser Basin: Phoenix Geyser erupted every 15,280–19,387 seconds (mean of 48 intervals = 17,794 seconds) for durations of ~1 hour.

Project title: Volcano Emissions

Principal investigator: Mr. Kenneth McGee
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Additional investigators: Terrence Gerlach, Michael Doukas

Objective: Research on various gas emissions from fumaroles, soils, and other sources within Yellowstone National Park for the purpose of defining a background level of emissions to which future measurements of anomalous degassing during volcanic or tectonic unrest could be compared.

Findings: Project is ongoing. Results to date indicate that gas measurements can be made in the air

above the park from an airborne platform.

Project title: Chloride Flux Monitoring

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Additional investigators: Daniel R. Norton, Smokey Sturtevant

Objective: To secure a long-term database of thermal activity in YNP.

Findings: Nineteen years of data on the chloride flux exiting YNP (which proxies for heat flow out of the Park) shows a decline of thermal discharge from the park of 10–12% over this time period. We believe that this decline may be related to the documented deflation of the Yellowstone caldera.

WATER QUALITY

Project title: Trophic Classification of Selected Lakes in Yellowstone National Park

Principal investigator: Dr. Woodruff Miller

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Objective: To evaluate the trophic state of lakes in Yellowstone National Park.

Findings: Lake: Trophic State; Lake South of Nymph Lake: Mesotrophic; Nymph Lake: Strongly Mesotrophic; South Twin Lake: Slightly Mesotrophic; North Twin Lake: Mesotrophic; Beaver Lake: Strongly Mesotrophic; Swan Lake: Mesotrophic; Harlequin Lake: Slightly Mesotrophic; Goose Lake: Slightly Mesotrophic; Blacktail Pond: Mesotrophic; Lake of the Woods: Strongly Mesotrophic; Hot Lake: Slightly Oligotrophic; Pool by Morning Glory Pool: Oligotrophic.

Nymph Lake is classified as Strongly Mesotrophic because of the high in-lake total phosphorus and should be of concern. Beaver Lake is also classified as Strongly Mesotrophic because of the high inflow total phosphorus and should also be of concern. While North Twin Lake has an overall classification of Mesotrophic, there should be some concern with the Hyper-eutrophic Vollenweider's model (high inflow TP) results.

Lake South of Nymph Lake, South Twin Lake, Swan Lake, Harlequin Lake, and Blacktail Pond are all Mesotrophic or Slightly Mesotrophic. These are healthy, appropriately productive lakes with no cause for concern at the present time.

The Strongly Mesotrophic State of Lake of the Woods was determined from only one month of data (one sample). Therefore, this lake needs further study in order to determine if it is of concern. Data are also scarce for Pool by Morning Glory Pool, Hot Lake, and Goose Lake. However, the available data for these lakes are fairly consistent, so these lakes should not be of concern for now.

Project title: Missouri–Madison Water Quality Monitoring Study

Principal investigator: Mr. Frank Pickett

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Additional investigators: Mary Bateson, Anthony Scotti, Thane Papke, Mike Ferris,
Ulrich Nuebel, Marcel van der Meer

Objective: Obtain baseline water quality data for use in assessing the quality of water in the mainstem Madison and discharges from PPL Montana Dams Hebgen and Madison and to provide information on New Zealand Mud Snail populations over time.

Findings: n/a

Project title: Yellowstone River Basin Study Unit National Water Quality Assessment

Principal investigator: Mr. Thomas Quinn

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Additional investigators: Gregory K. Boughton, Ron Zelt, David Peterson, Peter Wright

Objective: The overall goals of the NAWQA Program are to 1) describe current water quality conditions for a large part of the Nations freshwater streams and aquifers, 2) describe how water quality is changing over time, and 3) improve our understanding of the primary natural and human factors affecting the water quality.

Findings: Samples were collected at two sites during this reporting year, Soda Butte Creek, site 06187915, and at an Unnamed Tributary to Blacktail Deer Creek. At Soda Butte Creek water quality samples were collected for a variety of parameters such as common anions, nutrients, trace metals, and chlorophyll a. Samples from Soda Butte Creek were sent to the USGS National Laboratory in Lakewood, Colorado for analysis. Data collected in FY2001 will be published in the USGS Report, Wyoming Water Resources Data Report, and FY2001, which will be published in early 2002.

At Blacktail Deer Creek samples collected included water-quality, algae, macro-invertebrates, fish tissue and community, and habitat measurements. This data was collected in cooperation with the USEPA as part of the Western EMAP program. All data collected at Blacktail Deer Creek is available from the EPA.

Project title: Wyoming Reference Stream Project

Principal investigator: Mr. Jeremy ZumBerge
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Objective: The objective of the Wyoming Reference Stream Project is to collect water quality, habitat, and biological data at least-impacted stream sites within each ecoregion of Wyoming. Data from these reference sites is used as a benchmark against which the water quality, habitat, and biological condition of other sites are compared. Middle Creek was selected as a reference site for the Middle Rockies ecoregion and has been monitored annually since 1993.

Findings: Middle Creek data has been incorporated into a statewide database for use in development of biological criteria. This project is expected to continue for a minimum of several more years.

Project title: Reference Stream Monitoring—Long-term Trend Sites

Principal investigator: Mr. Jeremy ZumBerge
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Additional investigator: Laura Gianakos

Objective: Assess long-term trends in water quality and biological condition at a minimally impacted stream (Middle Creek) in the Middle Rockies ecoregion. These data assist in establishing baseline information that can be used to assess water quality and biological condition at other streams within the Middle Rockies ecoregion.

Findings: Middle Creek is one of several reference quality streams in the Middle Rockies ecoregion being monitored on an annual basis.

WETLANDS

Project title: Assessing Ecosystem Integrity: An Approach to Modeling Energy Flow

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Objective: To develop and test models of energy flow through wetland aquatic invertebrates. Then, to evaluate the use of energy flow in assessing ecosystem integrity.

Findings: Events beyond my control precluded sampling in 2001. Work on the project will begin again in 2002.

**Project title: Hydrogeomorphic Approach to the Assessment of Wetlands in
Yellowstone National Park**

Principal investigator: Mr. Chris Noble

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Objective: Collect data on undisturbed wetlands for characterization and scaling of variables for wetland assessment methods in the northern Rocky Mountains.

Findings: Data was collected at one slope wetland in the Lamar valley in July 2001.

Project title: Climate Effects on Small Mammals: A Multi-Scale Approach to the Study of Mammalian Response to Global Climate Change

Principal investigator: Ms. Kim O'Keefe

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Additional investigators: Kathleen Brizgys, Michael Milne

Objective: Our ability to predict the effects of global warming on biotic communities ultimately depends on our understanding of how individual species track specific climate variable at multiple scales. The purpose of this study is to gain insight into biotic response to climate change by characterizing the effects of climate on ground squirrel distribution, body size and population dynamics. Specifically, I hypothesize that temperature limits the distribution of *Spermophilus armatus*. To test this I will examine both present and historical patterns of distribution. First, I will use GIS data in conjunction with digitized locality data to perform statistical correlation analyses to determine if temperature correlates with the present distributional boundaries of the species. Second, to determine if there has been local extirpation and/or species replacement in response to the climate fluctuation of the late Holocene, I will perform phylogenetic analyses of ancient DNA sequences from specimens excavated from Lamar cave, a paleontological site in Yellowstone National Park. I also hypothesize that the effects of temperature will affect ground squirrel biology at the population level. I predict that in addition to range shifts, one of the ways in which ground squirrels respond to changes in temperature is by proximate and local changes in body size. I will test this in two ways. First I will examine the population level, phenotypic response of *S. armatus* to changes in the regional climate during the late Holocene by tracking changes in the body size of specimens from Lamar Cave. I will also study the distribution of body size along an elevational gradient. Changes in body size have direct and predictable effects on life history characteristics and thus on population dynamics. In order to gain insight into the mechanistic processes that explain these broader patterns of response I will develop and test a model that examines how the energetics of the individual are effected by the local thermal environment. Ground squirrel of the genus *Spermophilus* are particularly useful for studying biotic response to climate change because they are obligate hibernators and they show sensitivity to environment cues such as temperature. In addition, ground squirrels do not migrate therefore changes in abundance, distribution or the timing of life history events reflect response to local climate phenomena. Ground squirrels are also a vital link in terrestrial trophic interactions. As such, changes in ground squirrel abundance and distribution are likely to affect other species that prey on them. Thus ground squirrels may prove to be a useful indicator species

for tracking the effects of current climate change at the local level and for predicting the effects of climate change on the community.

Findings: As part of an effort to better understand how microclimate effects the body size and population density of ground squirrels we trapped, measured and marked individual ground squirrels from four sites along an elevational (and thus temperature) gradient. Our field research in Yellowstone National Park was conducted from July 1 to July 31, 2001. Three of our sites were within the boundaries of the park. At each site (worked sequentially) we set up a trapping grid of 150 ft x 245 ft and laid out 21 tomahawk live-traps. Each captured animal was given a unique ear tag number and dye mark on their pelage. The dye mark allowed us to determine whether we'd caught a particular animal using binoculars and thus determine how well we'd trapped the colony from afar. In addition, it allowed us to determine and record a recapture without having to re-handle the animal to examine its ear-tag number. Sex, estimated age and standard body size measurements including: body mass, total length, tail length, hind foot, ear from notch and zygomatic width were taken for each animal captured. In addition we visually mapped the positions of each burrow entrance on the grid to determine the burrow-density (BD) and better understand how well BD represents the total density of animals.

The following is a summary of our summer research with a brief description of our preliminary findings. Site one (Gardiner) is located about 0.5 miles up the old Gardiner Highway from the North Entrance gate. We caught and released 18 animals in 525 trapping hours. Site 2 (Lamar) is located about four miles south of the Slough Creek Campground on the northwest side of the Lamar River. We worked there from July 17–July 22. We trapped for a total of 211 trapping hours and captured and released 20 individuals. Site three (Lamar South) is located just across the Lamar River from Site 2 and it is approximately one mile north of the northeast entrance road. We worked there from July 23–July 25 and we trapped for a total of 173 trapping hours. We caught and released 23 individuals. Site four is outside of the park in the Shoshone National Forest. We caught 13 individuals in 320 trapping hours.

To compare the morphologic measurements between the sites I first separated the data by sex and age. I then performed a series of One-Way ANOVAs to test for significant differences in body size between the sites. Preliminary analysis of the morphologic data indicates that there is significant difference in the average body size of ground squirrels between the four sites with the largest animals occurring in the highest elevations (as predicted by Bergman's rule). Burrow density varies dramatically between sites but does not correlate with the number of individuals caught at each site. However I was unable to do a total count of all individuals at each site this season.

This report summarizes what I have found from the first of three planned field seasons. I will need to return this coming season to repeat the measurements and counts at each site. This time I hope to arrive just as the ground squirrels emerge from hibernation and then again as they immerge.

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